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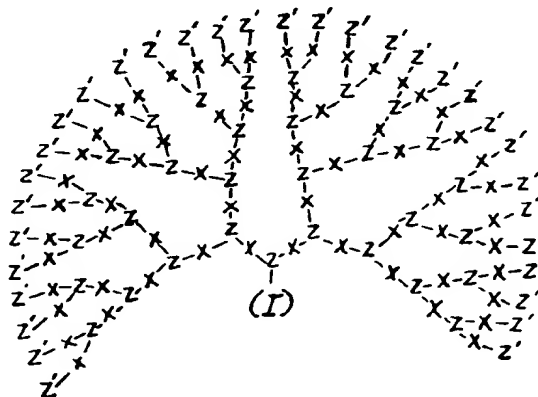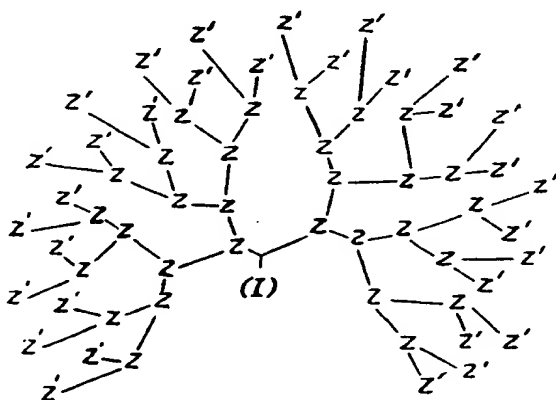
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(54) Title: STARBURST CONJUGATES

(57) Abstract

Starburst conjugates which are composed of at least one dendrimer in association with at least one unit of a carried agricultural material have been prepared. These conjugates have particularly advantageous properties due to the unique characteristics of the dendrimer.

STARBURST CONJUGATES

The present invention concerns the use of dense
star polymers as carriers for agricultural materials.
In recent years polymers referred to as dense star
polymers or starburst polymers have been developed. It

5   has been found that the size, shape and properties of
these dense star polymers or starburst polymers can be
molecularly tailored to meet specialized end uses.
Starburst polymers have significant advantages which

10 can provide a means for the delivery of high concen-
trations of carried material per unit of polymer, con-
trolled delivery, targeted delivery and/or multiple
species delivery or use.

15 In its broadest aspect, the present invention is
directed to polymer conjugate materials comprising
dense star polymers or starburst polymers associated
with desired agricultural materials (hereinafter these
polymer conjugates will frequently be referred to as

20 "starburst conjugates" or "conjugates"), processes for
preparing these conjugates, compositions

25

containing the conjugates, and methods of using the conjugates and compositions.

The conjugates of the present invention are suitable for use in a variety of applications where specific delivery is desired, and are particularly suited for the delivery of biologically active agents. In a preferred embodiment of the present invention, the starburst conjugates are comprised of one or more starburst polymers associated with one or more bioactive agents.

The starburst conjugates offer significant benefits over other carriers known in the art due to the advantageous properties of the starburst polymers. Starburst polymers exhibit molecular architecture characterized by regular dendritic branching with radial symmetry. These radially symmetrical molecules are referred to as possessing "starburst topology". These polymers are made in a manner which can provide concentric dendritic tiers around an initiator core. The starburst topology is achieved by the ordered assembly of organic repeating units in concentric, dendritic tiers around an initiator core; this is accomplished by introducing multiplicity and self-replication (within each tier) in a geometrically progressive fashion through a number of molecular generations. The resulting highly functionalized molecules generations have been termed "dendrimers" in deference to their branched (tree-like) structure as well as their oligomeric nature. Thus, the terms starburst oligomer and starburst dendrimer are encompassed within the term starburst polymer. Topological polymers, with size and shape controlled domains, are dendrimers that are covalently bridged

through their reactive terminal group which are referred to as "starburst bridged dendrimers", which is also encompassed within the term starburst polymer.

5   The following description of the figures aids in understanding the present invention.

Figure 1 depicts various generations of starburst dendrimers.

10   Figure 2A depicts a dendrimer having unsymmetrical (unequal) branch junctures.

Figure 2B depicts a dendrimer having symmetrical (equal) branch junctures.

15   Figure 3 shows carbon-13 spin lattice relaxation times (T_1) for aspirin incorporated into various dendrimer generations. (Example 4)

20   The starburst polymers are illustrated by Figure 1 wherein ⓘ represents an initiator core (in this figure a tri-functional initiator core, shown by the far left drawing); Z represents a terminal group, shown in the first instance by the second drawing from the left, referred to as a starbranched oligomer; A, B,

25   C, D, and E represent particular molecular generations of starburst oligomers, called dendrimers; and $(A)_n$, $(B)_n$, $(C)_n$, $(D)_n$, and $(E)_n$ represent starburst bridged dendrimers.

30   The starburst dendrimers are unimolecular assemblages that possess three distinguishing architectural features, namely, (a) an initiator core,

35   (b) interior layers (generations, G) composed of repeating units, radially attached to the initiator

core, and (c) an exterior surface of terminal
functionality (i.e., terminal functional groups)
attached to the outermost generation. The size and
shape of the starburst dendrimer molecule and the
5 functional groups present in the dendrimer molecule can
be controlled by the choice of the initiator core, the
number of generations (i.e., tiers) employed in
creating the dendrimer, and the choice of the repeating
units employed at each generation. Since the
10 dendrimers can be readily isolated at any particular
generation, a means is provided for obtaining
dendrimers having desired properties.

The choice of the starburst dendrimer
15 components affects the properties of the dendrimers.
The initiator core type can affect the dendrimer shape,
producing (depending on the choice of initiator core),
for example, spheroid-shaped dendrimers, cylindrical or
rod-shaped dendrimers, ellipsoid-shaped dendrimers, or
20 mushroom-shaped dendrimers. Sequential building of
generations (i.e., generation number and the size and
nature of the repeating units) determines the
dimensions of the dendrimers and the nature of their
25 interior.

Because starburst dendrimers are branched
polymers containing dendritic branches having
functional groups distributed on the periphery of the
30 branches, they can be prepared with a variety of
properties. For example, starburst dendrimers, such as
those depicted in Figure 2A and Figure 2B can have
distinct properties due to the branch length. The
dendrimer type shown in Figure 2A possesses
35 unsymmetrical (unequal segment) branch junctures,
exterior (i.e., surface) groups (represented by Z'),

interior moieties (represented by Z) but much less
internal no internal void space. The dendrimer type
shown in Figure 2B possesses symmetrical (equal
segment) branch junctures with surface groups
5   (represented by Z'), two different interior moieties
(represented respectively by X and Z) with interior
void space which varies as a function of the generation
(G). The dendrimers such as those depicted in Figure
2B can be advanced through enough generations to
10 totally enclose and contain void space, to give an
entity with a predominantly hollow interior and a
highly congested surface. Also, starburst dendrimers,
when advanced through sufficient generations exhibit
"starburst dense packing" where the surface of the
15 dendrimer contains sufficient terminal moieties such
that the dendrimer surface becomes congested and
encloses void spaces within the interior of the
dendrimer. This congestion can provide a molecular
level barrier which can be used to control diffusion of
20 materials into or out of the interior of the dendrimer.

Surface chemistry can be controlled in a
predetermined fashion by selecting a repeating unit
25 which contains the desired chemical functionality or by
chemically modifying all or a portion of the surface
functionalities to create new surface functionalities.
These surfaces may either be targeted toward specific
sites or made to resist uptake by cells. In an
30 alternative use of the dendrimers, the dendrimers can
themselves be linked together to create polydendric
moieties (starburst "bridged dendrimers") which are
also suitable as carriers.

35 In addition, the dendrimers can be prepared so
as to have deviations from uniform branching in

particular generations, thus providing a means of
adding discontinuities (i.e., deviations from uniform
branching at particular locations within the dendrimer)
and different properties to the dendrimer.

5

The starburst polymers employed in the
starburst conjugates of the present invention can be
prepared according to methods known in the art, for
example, U. S. Patent 4,587,329.

10

Dendrimers can be prepared having highly
uniform size and shape and most importantly allow for a
greater number of functional groups per unit of surface
area of the dendrimer, and can have a greater number of

15 functional groups per unit of molecular volume as
compared to other polymers which have the same
molecular weight, same core and monomeric components
and same number of core branches as the starburst
polymers. The increased functional group density of

20 the starburst polymers may allow a greater quantity of
material to be carried per dendrimer.

An analogy can be made between early generation
starburst dendrimers (i.e., generation = 1-7) to

25 classical spherical micelles. The dendrimer – micelle
analogy was derived by comparing features which they
had in common such as shape, size and surface.

30

35

TABLE I

Parameter	Regular Classical Micelles	Starburst Dendrimers
Shape	Spherical	Spherical
Size (diameter)	20-60Å	17-67Å
Surface Aggregation Numbers	4-202	Z=6-192 (Generation = 2-7)
Area/Surface Group (Å)	130-80Å^2	127-75Å^2

Z is the number of surface groups; $1Å = 10^{-1}$ nm; $1Å^2 = 10^{-2}$ nm^2.

In Table I, the shape was verified by scanning transmission electron micrographs (STEM) microscopy and intrinsic viscosity (η) measurements. The size was verified by intrinsic viscosity (η) and size exclusion chromatography (SEC) measurements. The surface aggregation numbers were verified by titremetry and high field NMR. The area/surface group was calculated from SEC hydrodynamic measurements.

The first five generations of starburst polyamidoamine (PAMAM) dendrimers are microdomains which very closely mimic classical spherical micelles in nearly every respect (i.e shape, size, number of surface groups, and area/surface group). A major difference, however, is that they are covalently fixed and robust compared to the dynamic equilibration of nature of micelles. This difference is a significant

advantage when using these microdomains as controlled
delivery prototypes or encapsulation devices.

5

As further concentric generations are added
beyond five, congestion of the surface occurs. This
congestion can lead to increased barrier
characteristics at the surface and manifests itself as
a smaller surface area per head (surface) group as
shown in Table II.

10

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Table II
PAMAM Dendrimer Features vs. Generation

Generations	1	2	3	4	5	6	7	8	9
# of surface groups, Z	3	6	12	24	48	96	192	384	768
Molecular wt.	275	875	2411	5147	10,619	21,563	43,541	87,227	174,779
Diameter* measured SEC	10.4Å	15.8Å	22Å	31Å	40Å	53Å	67Å	76Å	88Å
Surface area per dendrimer	366Å^2	783Å^2	1519Å^2	3018Å^2	5024Å^2	8,820Å^2	14,096Å^2	18,136Å^2	36,083Å^2
Surface area per Z group	122Å^2	131Å^2	127Å^2	126Å^2	104Å^2	92Å^2	73Å^2	47Å^2	32Å^2
Distance between Z groups	12.4Å	12.8Å	12.7Å	12.6Å	11.5Å	10.8Å	9.8Å	7.75Å	6.28Å
Void Volume	311.6Å^3	1,470.2Å^3	4,737.9Å^3	11,427.0Å^3	---	---	---	---	---

* Hydrodynamic diameters determined by size exclusion chromatogaphy measurements calibrated against monodisperse ($\underline{Mw} = 1.02$) polyethyleneoxide standards.
$\underline{}$
Mn

$1Å = 10^{-1}$ nm; $1Å^2 = 10^{-2}$ nm^2; $1 Å^3 = 10^{-3}$ nm^3.

For example, amine terminated generations 5.0, 6.0, 7.0, 8.0 and 9.0 have decreased surface areas of 104, 92, 73, 47 and 32$Å^2$ per Z group, respectively. This characteristic corresponds to a transition from a

5   less congested micelle-like surface to a more congested bilayer/monolayer barrier-like surface normally associated with vesicles (liposomes) or Langmuir-Blodgett type membranes.

10   If this surface congestion is indeed occurring, the change in physical characteristics and morphology should be observed as the generations increase from the intermediate generations (6-8) to the more advanced generations (9 or 10). The scanning transmission

15   electon micrographs (STEM) for generations = 7.0, 8.0 and 9.0 were obtained after removing the methanol solvent from each of the samples to provide colorless, light yellow solid films and staining with osmium tetraoxide. The morphological change predicted

20   occurred at the generation, G = 9.0 stage. The microdomains at generation = 9.0 measure about 33$Å$ in diameter and are surrounded by a colorless rim which is about 25$Å$ thick. Apparently, methanolic solvent has

25   been entrapped within the 25$Å$ outer membrane-like barrier to provide the dark stained interior. Thus, at generation = 9.0, the starburst PAMAM is behaving topologically like a vesicle (liposome). However, this starburst is an order of magnitude smaller and very

30   monodispersed compared to a liposome. Consequently, the present dendrimers can be used to molecularly encapsulate solvent filled void spaces of as much diameter as about 33$Å$ (volume about 18,000$Å^3$) or more.

35   These micelle sized prototypes appear to behave like a covalently fixed liposome in this advanced generation

stage. These prototypes may have additional capability as carriers or as delivery agents.

Since the number of functional groups on the dendrimers can be controlled on the surface and within the interior, it also provides a means for controlling the amount of agricultural material to be delivered per dendrimer. In a particularly preferred embodiment of the present invention the dendrimers are targeted carriers of bioactive agents capable of delivering bioactive agent to a particular target organism such as a plant or pest or to a particular determinant or locus in a target organism. Dendrimers suitable for use in the conjugates of the present invention include the dense star polymers or starburst polymers described in U. S. Patents 4,507,466, 4,558,120, 4,568,737 and 4,587,329.

In particular, the present invention concerns a starburst conjugate which comprises at least one starburst polymer associated with at least one carried agricultural material. Starburst conjugates included within the scope of the present invention include those represented by the formula:

$$(P)_x * (M)_y \qquad\qquad (I)$$

wherein each P represents a dendrimer;

x represents an integer of 1 or greater;

each M represents a unit (for example, a molecule, atom, ion, and/or other basic unit) of a carried agricultural material, said carried agricultural material can be the same carried agricultural material

or a different carried agricultural material,
preferably the carried material is a bioactive agent;

y represents an integer of 1 or greater; and

5 * indicates that the carried material is associated
with the dendrimer.

Preferred starburst conjugates of formula (I)
are those in which M is a pesticide, radionuclide,
10 chelant, toxin, or signal generator, signal reflector,
or signal absorber; particularly preferred are those in
which x=1, and y=2 or more.

Also included are starburst conjugates of
15 formula (I) wherein the starburst dendrimers are
covalently linked together, optionally via linking
groups, so as to form polydendric assemblages (i.e.,
where x>1). · Use of these starburst bridged dendrimers
include topical controlled release agents.
20

As used herein, "associated with" means that
the carried material(s) can be encapsulated or
entrapped within the core of the dendrimer, dispersed
partially or fully throughout the dendrimer, or
25 attached or linked to the dendrimer, or any combination
thereof. The association of the carried material(s)
and the dendrimer(s) may optionally employ connectors
and/or spacers to facilitate the preparation or use of
30 the starburst conjugates. Suitable connecting groups
represented by C', are groups which link a targeting
director (i.e., T) to the dendrimer (i.e., P) without
significantly impairing the effectiveness of the
director or the effectiveness of any other carried
35 material(s) (i.e., M) present in the starburst
conjugate. These connecting groups may be cleavable or

non-cleavable and are typically used in order to avoid
steric hindrance between the target director and the
dendrimer, preferably the connecting groups are stable
(i.e., non-cleavable). Since the size, shape and
5   functional group density of the dense star dendrimers
can be rigorously controlled, there are many ways in
which the carried material can be associated with the
dendrimer. For example, (a) there can be covalent,
coulombic, hydrophobic, or chelation type association
10 between the carried material(s) and entities, typically
functional groups, located at or near the surface of
the dendrimer; (b) there can be covalent, coulombic,
hydrophobic, or chelation type association between the
carried material(s) and moieties located within the
15 interior of the dendrimer; (c) the dendrimer can be
prepared to have an interior which is predominantly
hollow allowing for physical entrapment of the carried
materials within the interior (void volume); wherein
20 the release of the carried material can optionally be
controlled by congesting the surface of the dendrimer
with diffusion controlling moieties; or (d) various
combinations of the aforementioned phenomena can be
employed.
25

     Dendrimers, herein represented by "P", include
the dense star polymers described in U. S.
Patents 4,507,466; 4,558,120; 4,568,737 or 4,587,329.

30   Carried agricultural materials, including the
term "agricultural materials", herein represented by
"M", which are suitable for use in the starburst
conjugates include any materials for _in vivo_ or _in
vivtro_ treatment, diagnosis, or application to plants
35 and non-mammals (including microorganisms) which can be
associated with the starburst dendrimer without

appreciably disturbing the physical integrity of the
dendrimer. For example, carried materials like toxins
such as diphtheria toxin, gelonin, exotoxin A, abrin,
modeccin, ricin, or toxic fragments thereof; metal ions
such as the alkali and alkaline-earth metals;
radionuclides such as those generated from actinides or
lanthanides or other similar transition elements or
from other elements, such as ^{67}Cu, ^{90}Y, ^{111}In, ^{131}I,
^{186}Re, ^{105}Rh, ^{99m}Tc, ^{67}Ga, ^{153}Sm, ^{159}Gd, ^{175}Yb, ^{177}Lu,
^{88}Y, ^{166}Ho, ^{115m}In, ^{109}Pd, ^{82}Rb, ^{194}Ir, ^{140}Ba, ^{149}Pm,
^{199}Au, ^{140}La, and ^{188}Re; signal generators such as
fluorescing entities; signal reflectors such as
paramagnetic entities; signal absorbers such as
electron beam opacifiers; hormones; biological response
modifiers such as interleukins, interferons, viruses
and viral fragments; pesticides, including
antimicrobials, algicides, arithelmetics, acaricides,
insecticides, attractants, repellants, herbicides
and/or fungicides such as acephate, acifluorfen,
alachlor, atrazine, benomyl, bentazon, captan,
carbofuran, chloropicrin, chlorpyrifos, chlorsulfuron
cyanazine, cyhexatin, cypermetrin, 2,4-dichloro-
phenoxyacetic acid, dalapon, dicamba, diclofop methyl,
diflubenzuron, dinoseb, endothall, ferbam, fluazifop,
glyphosate, haloxyfop, malathion, naptalam,
pendimethalin, permethrin, picloram, propachlor,
propanil, sethoxydin, temephos, terbufos, trifluralin,
triforine, zineb, and the like. Carried agricultural
materials include scavenging agents such as chelants,
chelated metal (whether or not they are radioactive),
or any moieties capable of selectively scavenging
therapeutic or diagnostic agents.

Preferably the carried materials are bioactive agents. As used herein, "bioactive" refers to an active entity such as a molecule, atom, ion and/or other entity which is capable of detecting,

5 identifying, inhibiting, treating, catalyzing, controlling, killing, enhancing or modifying a targeted entity such as a protein, glycoprotein, lipoprotein, lipid, a targeted cell, a targeted organ, a targeted organism [for example, a microorganism, plant, or animal

10 (excluding mammals)] or other targeted moiety.

The starbursts conjugates of formula (I) are prepared by reactive P with M, usually in a suitable solvent, at a temperature which facilitates the

15 association of the carried material (M) with the starburst dendrimer (P).

Suitable solvents are solvents in which P and M are at least partially miscible and inert to the

20 formation of the conjugate. If P and M are at least partially miscible with each other, no solvent may be required. When desired, mixtures of suitable solvents can be utilized. Examples of such suitable solvents are water, methanol, ethanol, chloroform, acetonitrile,

25 toluene, dimethylsulfoxide and dimethylformamide.

The reaction condition for the formation of the starburst conjugate of formula (I) depends upon the particular dendrimer (P), the carried agricultural

30 material (M), and the nature of the bond (*) formed. For example if P is the PEI (polyethyleneimine) starburst dendrimer with a methylene carboxylate surface, M is a radionuclide, e.g. yttrium, then the

35 reaction is conducted at room temperature in water. Typically, the temperature can range from room

temperature to reflux. The selection of the particular
solvent and temperature will be apparent to one skilled
in the art.

5   The ratio of M:P will depend on the size of the
dendrimer and the amount of carried material. For
example, the molar ratio (ratio of moles) of any ionic
M to P is usually 0.1−1,000:1, preferably 1−50:1, and
more preferably 2−6:1. The weight ratio of any
10 pesticide or toxin M to P is usually 0.1−5:1, and
preferably 0.5−3:1.

   When M is a radionuclide, there are three ways
the starburst conjugate can be prepared, namely: (1) P
15 can be used as a chelant. For example a
methylenecarboxylate surface PEI or PAMAM will chelate
a metal such as yttrium or indium. (2) A chelate can
be covalently bonded to P. For example, an amine
terminated PEI starburst dendrimer can be reacted with
20 1−(p−isothiocyanatobenzyl)diethylenetriaminepenta-
acetic acid and then chelated, or a complex such as
rhodium chloride chelated with isothiocyanatobenzyl-
2,3,2-tet can be reacted. (3) A prechelated
radionuclide can be associated with P by hydrophobic or
25 ionic intereaction.

   Other starburst conjugates are those conjugates
which contain a target director (herein designated as
"T") and which are represented by the formula:
30

$$(T)_e * (P)_x * (M)_y \qquad\qquad (II)$$
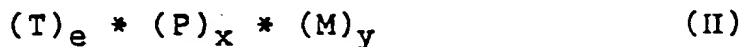
35 wherein

each T represents a target director;

e represents an integer of 1 or greater; and

P, x, *, M, and y are as previously defined herein.

Preferred among the starburst conjugates of formula (II) are those in which M is a pesticide, radionuclide, chelator, chelated metal, toxin, signal generator, signal reflector, or signal absorber. Also preferred are those conjugates in which e=1 or 2; and those in which x=1 and y=2 or more. Particularly preferred conjugates are those in which x=1, e=2, y=2 or more and M and T are associated with the polymer via the same or different connectors.

The starburst conjugates of formula (II) are prepared either by forming T*P and then adding M or by forming P*M and then adding T. Either reaction scheme is conducted at temperatures which are not detrimental to the particular conjugate component and in the presence of a suitable solvent when required. To control pH, buffers or addition of suitable acid base is used. The reaction conditions are dependent on the type of association formed (*), the starburst dendrimer used (P), the carried agricultural material (M), and the target director (T). Alternatively, P and M can be chelated, usually in water, before conjugation to T. The conjugation with T is carried out in a suitable buffer.

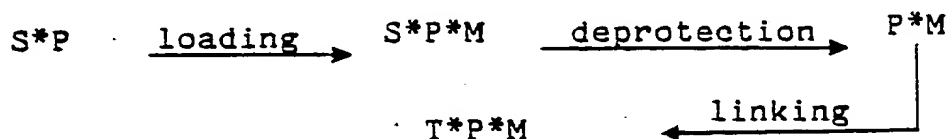The ratio of T:P is preferably 1:1. The ratio of M:P will be as before.

Target directors capable of targeting the starburst conjugates are entities which when used in

the starburst conjugates of the present invention result in at least a portion of the starburst conjugates being delivered to a desired target (for example, a protein, glycoprotein, lipoprotein, lipid, a targeted cell, a targeted organism or other targeted moiety) and include hormones, biological response modifiers, chemical functionalities exhibiting target specificity, and the like.

In the absence of a target director (or in the presence of a target director if desired), due to the number of functional groups which can be located at or near the surface of the dendrimer, all or a substantial portion of such functional groups can be made anionic, cationic, hydrophobic or hydrophilic to effectively aid delivery of the starburst conjugate to a desired target of the opposite charge or to a hydrophobic or hydrophilic compatible target.

Preparation of the conjugates of formula (II) using a P with a protected handle (S) is also intended as a process to prepare the conjugates of formula (II). The reaction scheme is shown below:

$$S*P \quad \xrightarrow{\text{loading}} \quad S*P*M \quad \xrightarrow{\text{deprotection}} \quad P*M$$

$$T*P*M \quad \xleftarrow{\text{linking}}$$

where

S*P   represents the protected dendrimer;

S*P*M   represents the protected dendrimer conjugated with m;

P*M   represents the dendrimer conjugated
with M (starburst conjugate);

T*P*M   represents the starburst conjugates
liked to the target director.

Suitable solvents can be employed which do not
effect P*M. For example when S is t-butoxycarbamate, S
can be removed by aqueous acid.

The starburst conjugates can be used for a
variety of _in vivo_ and _in vitro_ diagnostic applications
pertaining to plants and non-mammals, such as
radioimmunoassays, electron microscopy, enzyme linked
immunosorbent assays, nuclear magnetic resonance
spectroscopy, contrast imaging, and immunoscintography,
in analytical applications; and in biological control
applications as a means of delivering pesticides such
as herbicides, insecticides, fungicides, repellants,
attractants, repellants, attractants, antimicrobials or
other toxins, or used as starting materials for making
other useful agents.

The present invention is also directed to
starburst conjugate compositions in which the starburst
conjugates are formulated with other suitable vehicles
useful in agriculture such as on crops, fallow land, or
as pesticides, or in treatment of or _in vivo_ or _in
vitro_ testing of non-mammals. The starburst conjugate
compositions may optionally contain such other active
ingredients, additives and/or diluents.

An agriculturally acceptable carrier or diluent
which may also be present with one or more starburst
conjugates of the present invention includes those
carriers or diluents customarily used in granular

formulations, emulsifible concentrates, solutions, or
suspensions such as, for example, toluene, xylene,
benzene, phenol, water, methane, hydrocarbons,
naphthalene and others.

5   The preferred starburst polymer for use in the
starburst conjugates of the present invention is a
polymer that can be described as a starburst having at
least one branch (hereinafter called a core branch),
10   preferably two or more branches, emanating from a core,
said branch having at least one terminal group provided
that (1) the ratio of terminal groups to the core
branches is more than one, preferably two or greater,
(2) the density of terminal groups per unit volume in
15   the polymer is at least 1.5 times that of an extended
conventional star polymer having similar core and
monomeric moieties and a comparable molecular weight
and number of core branches, each of such branches of
the extended conventional star polymer bearing only one
20   terminal group, and (3) a molecular volume that is no
more than about 80 percent of the molecular volume of
said extended conventional star polymer as determined
by dimensional studies using scaled Corey-Pauling
25   molecular models. As used herein, the term "dense" as
it modifies "star polymer" or "dendrimer" means that it
has a smaller molecular volume than an extended
conventional star polymer having the same molecular
weight. The extended conventional star polymer which
30   is used as the base for comparison with the starburst
polymer is one that has the same molecular weight, same
core and monomeric components and same number of core
branches as the starburst polymer. By "extended" it is
meant that the individual branches of the conventional
35   star polymer are extended or stretched to their maximum

length, e.g., as such branches exist when the star
polymer is completely solvated in an ideal solvent for
the star polymer. In addition while the number of
terminal groups is greater for the starburst polymer
5   molecule than in the conventional star polymer
molecule, the chemical structure of the terminal groups
is the same.

Dendrimers used in the conjugates of the
10   present invention can be prepared by processes known in
the art. The above dendrimers, the various coreactants
and core compounds, and process for their preparation
can be as defined in U. S. Patent 4,587,329.

15   The starburst dendrimers, for use in the
starburst conjugates of the present invention, can have
terminal groups which are sufficiently reactive to
undergo addition or substitution reactions. Examples
of such terminal groups include amino, hydroxy,
20   mercapto, carboxy, alkenyl, allyl, vinyl, amido, halo,
urea, oxiranyl, aziridinyl, oxazolinyl, imidazolinyl,
sulfonato, phosphonato, isocyanato and isothiocyanato.
The terminal groups can be modified to make them
biologically inert. The dendrimers differ from
25   conventional star or star-branched polymers in that the
dendrimers have a greater concentration of terminal
groups per unit of molecular volume than do
conventional extended star polymers having an
30   equivalent number of core branches and an equivalent
core branch length. Thus, the density of terminal
groups per unit volume in the dendrimer usually is at
least about 1.5 times the density of terminal groups in
the conventional extended star polymer, preferably at
35   least 5 times, more preferably at least 10 times, most
preferably from 15 to 50 times. The ratio of terminal

groups per core branch in the starburst dendrimer is
preferably at least 2, more preferably at least 3, most
preferably from 4 to 1024. Preferably, for a given
polymer molecular weight, the molecular volume of the
5   starburst dendrimer is less than 70 volume percent,
more preferably from 16 to 60, most preferably from 7
to 50 volume percent of the molecular volume of the
conventional extended star polymer.

10   Preferred starburst dendrimers for use in the
starburst conjugates of the present invention are
characterized as having a univalent or polyvalent core
that is covalently bonded to dendritic branches. Such
ordered branching can be illustrated by the following
15   sequence wherein G indicates the number of generations:
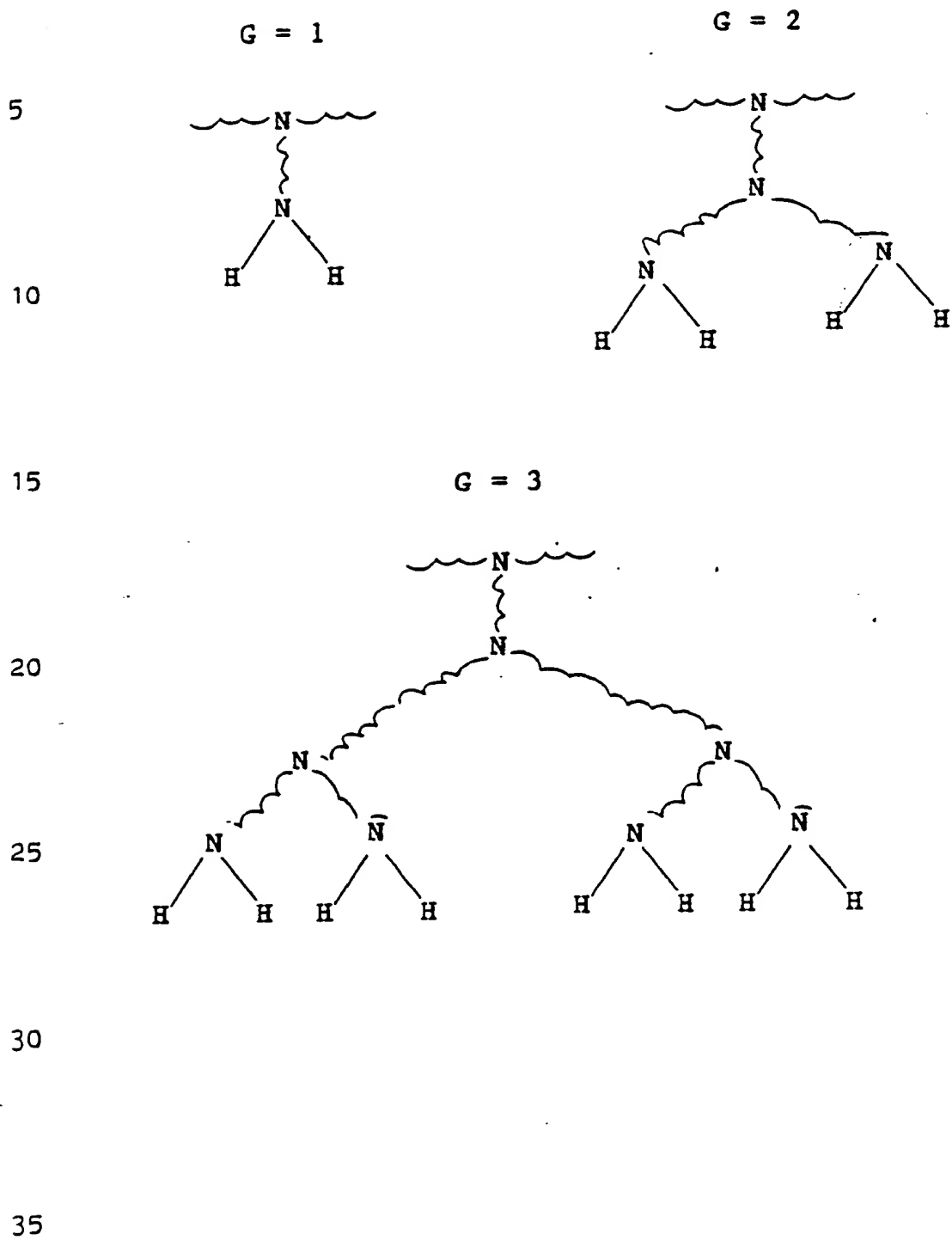
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25

30

35

G = 1

G = 2
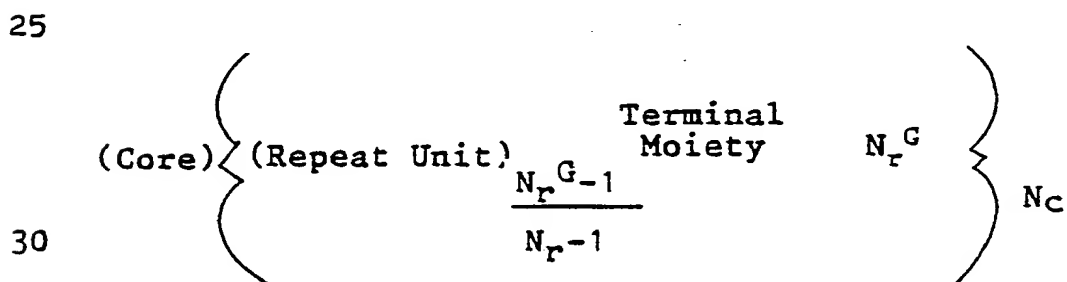
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10

15   G = 3

20

25

30

35

Mathematically, the relationship between the
number (#) of terminal groups on a dendritic branch and
the number of generations of the branch can be
represented as follows:

5

$$\text{\# of terminal groups per dendritic branch} = \frac{N_r{}^G}{2}$$

wherein G is the number of generations and N_r
10 is the repeating unit multiplicity which is at least 2
as in the case of amines. The total number of terminal
groups in the dendrimer is determiend by the following:

15           $$\text{\# of terminal groups per dendrimer} = \frac{N_c N_r{}^G}{2}$$

wherein G and N_r are as defined before and N_c
20 represents the valency (often called core
functionality) of the core compound. Accordingly, the
dendrimers of this invention can be represented in its
component parts as follows:

25

$$(\text{Core})\left\{ (\text{Repeat Unit})_{\frac{N_r{}^{G-1}}{N_r-1}} \quad \overset{\text{Terminal}}{\underset{}{\text{Moiety}}} \quad N_r{}^G \right\}_{N_c}$$

30

wherein the Core, Terminal Moiety, G and N_c are as
defined before and the Repeat Unit has a valency or
35

functionality of $N_r + 1$ wherein N_r is as defined before.

A copolymeric dendrimer which is a preferred dendrimer for the purposes of this invention is a unique compound constructed of polyfunctional monomer units in a highly branched (dendritic) array. The dendrimer molecule is prepared from a polyfunctional initiator unit (core compound), polyfunctional repeating units and terminal units which may be the same or different from the repeating units. The core compound is represented by the formula Ⓘ $(Z^C)_{Nc}$ wherein Ⓘ represents the core, Z^C represents the functional groups bonded to Ⓘ and Nc represents the core functionality which is preferably 2 or more, most preferably 3 or more. Thus, the dendrimer molecule comprises a polyfunctional core Ⓘ bonded to a number (N_c) of functional groups, Z^C, each of which is connected to the monofunctional tail of a repeating unit, $X^1Y^1(Z^1)_{N^1}$, of the first generation and each of the Z groups of the repeating unit of one generation is bonded to a monofunctional tail of a repeating unit of the next generation until the terminal generation is reached.

In the dendrimer molecule, the repeating units are the same within a single generation, but may differ from generation to generation. In the repeating unit, $X^1Y^1(Z^1)_{N^1}$, X^1 represents the monofunctional tail of the first generation repeating unit, Y^1 represents the moiety constituting the first generation, Z^1 represents the functional group of the polyfunctional head of the repeating unit of the first generation and may be the same as or different from the functional groups of the core compound, Ⓘ$(Z^C)_{Nc}$, or other generations; and N^1

is a number of 2 or more, most preferably 2, 3 or 4,
which represents the multiplicity of the polyfunctional
head of the repeating unit in the first generation.
Generically, the repeating unit is represented by the

5 formula $X^i Y^i (Z^i)_{N^i}$ wherein "i" represents the
particular generation from the first to the t-1
generation. Thus, in the preferred dendrimer molecule,
each Z^1 of the first generation repeating unit is
connected to an X^2 of a repeating unit of the second

10 generation and so on through the generations such that
each Z^i group for a repeating unit $X^i Y^i (Z^i)_{N^i}$ in
generation number "i" is connected to the tail (X^{i+1})
of the repeating unit of the generation number "i+1".
The final or terminal of a preferred dendrimer molecule

15 comprises terminal units, $X^t Y^t (Z^t)_{N^t}$ wherein t
represents terminal generation and X^t, Y^t, Z^t and N^t
may be the same as or different from X^i, Y^i, Z^i and N^i
except that there is no succeeding generation connected
to the Z^t groups and N^t may be less than two, e.g.,

20 zero or one. Therefore the preferred dendrimer has a
molecular formula represented by

2.

$$\left(\text{(I)}(Z^c)_{N_c}\right)\left\{\left(X^i\ Y^i(Z^i)_{N^i}\right)^{i-1}_{\substack{N_c \Pi N^n \\ n\ \text{is}\ 1}}\right\}\left(X^t Y^t(Z^t)_{N^t}\right)^{t-1}_{\substack{N_c \Pi N^n \\ n\ \text{is}\ 1}}$$

3

where i is 1 to t-1

35

wherein the symbols are as previously defined. The π function is the product of all the values between its defined limits. Thus

5
$$\prod_{n=1}^{i-1} N^n = (N^1)(N^2)(N^3)\cdots(N^{i-2})(N^{i-1})$$

which is the number of repeat units, $X^i Y^i (Z^i)_{N^i}$, comprising the ith generation of one dendritic branch

10 and when i is 1, then

$$\prod_{n=1} \pi^0 = 1 \quad .$$

15

'In copolymeric dendrimers, the repeat unit for one generation differs from the repeat unit in at least one

20 other generation. The preferred dendrimers are very symmetrical as illustrated in structural formulas described hereinafter. Preferreddendrimers may be converted to functionalized dendrimers by contact with another reagent. For example, conversion of hydroxyl

25 in the terminal generation to ester by reaction with an acid chloride gives an ester terminally functionalized dendrimer.   This functionalization need not be carried out to the theoretical maximum as defined by the number of available functional groups and, thus, a

30 functionalized dendrimer may not have high symmetry or a precisely defined molecular formula as is the case with the preferred dendrimer.

35 In a homoplymer dendrimer, all of the repeat units, $X^i Y^i (Z^i)_{N^i}$, are identical. Since the values of

all N^i are equal (defined as N_r), the product function representing the number of repeat units reduces to a simple exponential form. Therefore, the molecular formula may be expressed in simpler form as

$$\left(\textcircled{I}(Z^c)_{N_c}\right)\left\{\left(X^i \ Y^i \ (Z^i)_{N^i}\right)_{N_c N_r^{i-1}}\right\}\left(X^t Y^t (Z^t)_{N^t}\right)_{N_c N_r^{t-1}}$$

where i = 1 to t-1

This form still shows the distinction between the different generations i, which each consist of $N_c N_r^{(i-1)}$ repeating units, $X^i Y^i (Z^i)_{N^i}$. Combining the generations into one term gives:
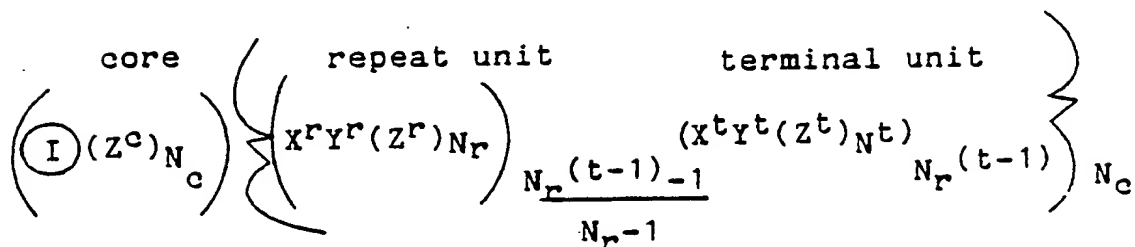
$$\left(\textcircled{I}(Z^c)_{N_c}\right)\left(X^rY^r(Z^r)_{N_r}\right)_{N_c}{}^{\frac{N_r{}^{(t-1)}-1}{N_r-1}}\left(X^tY^t(Z^t)_{N^t}\right)_{N_cN_r{}^{t-1}}$$

or

$$\left(\overset{\text{core}}{\textcircled{I}(Z^c)_{N_c}}\right)\left\{\overset{\text{repeat unit}}{\left(X^rY^r(Z^r)_{N_r}\right)_{\frac{N_r{}^{(t-1)}-1}{N_r-1}}}\overset{\text{terminal unit}}{\left(X^tY^t(Z^t)_{N^t}\right)_{N_r{}^{(t-1)}}}\right\}_{N_c}$$

wherein $X^rY^r(Z^r)_{N_r}$ is the repeating unit which is used
in all generations i.

20

Consequently, if a polymer compound will fit
into these above formulae, then the polymer is a
starburst polymer. Conversely, if a polymer compound
will not fit into these above formulae, then the

25   polymer is not a starburst polymer. Also, to determine
whether a polymer is a starburst polymer, it is not
necessary to know the process by which it was prepared,
but only whether it fits the formulae. The formulae

30   also demonstrate the generations (G) or tiering of
dendrimers.

Clearly, there are several ways to determine
the ratio of agent (M) to dendrimer (P) which depend

35   upon how and where the association of P*M occurs. When
there is interior encapsulation, the weight ratio of

M:P usually is 10:1, preferably 8:1, more preferably
5:1, most preferably 3:1. The ratio can be as low as
0.5:1 to 0.1:1. When interior stoichiometry is used,
the weight ratio of M:P is the same as for interior
encapsulation. When exterior stoichiometry is
determined, the mole/mole ratio of M:P given by the
following formulae:

	M	:	P
(A)	$5\ N_c N_t N_r{}^{G-1}$		1
(B)	$3\ N_c N_t N_r G^{-1}$		1
(C)	$1\ N_c N_t N_r{}^{G-1}$		1

where N_c means the core multiplicity, N_t means the
terminal group multiplicity, and N_r means branch
juncture multiplicity. The $N_c N_t N_r G^{-1}$ term will result
in the number of Z groups. Thus, for example, (A)
above may result when proteins, enzymes or highly
charged molecules are on the surface; (B) above when it
is 2,4-D or octanoic acid; (C) above when converting
surface ester groups to carboxylate ions or groups.

Of course other structures of various
dimensions can be readily prepared by one skilled in
the art by appropriately varying the dendrimer
components and number of generations employed. The
dimensions of the dendrimers are significant in that
they are small. A linear polymer of comparable
molecular weight would have a radius of gyration, (in
its fully extended form), that would be much larger
than the same molecular weight dendrimer.

Linking target directors to dendrimers is another aspect of the present invention. In preferred embodiments of the present invention, a reactive functional group such as a carboxyl, sulfhydryl,

5   reactive aldehyde, reactive olefinic derivative, isothiocyanato, isocyanato, amino, reactive aryl halide, or reactive alkyl halide can conveniently be employed on the dendrimer. The reactive functional groups can be introduced to the dendrimer using known

10   techniques, for example:

(1) Use of a heterofunctional initiator (as a starting material for synthesizing the dendrimer) which has incorporated into it functional groups of different

15   reactivity. In such heterofunctional initiator at least one of the functional groups will serve as an initiation site for dendrimer formation and at least one of the other functional groups will be available for linking to a target director but unable to initiate

20   dendrimer synthesis. For example, use of protected aniline to allow further modification of NH_2 groups within the molecule without reacting the aniline NH_2.

25   The functional group which will be available for linking to a target director may be part of the initiator molecule in any one of three forms namely:

(a) In the form in which it will be used for linking with the target director. This is

30   possible when none of the synthetic steps involved in the dendrimer synthesis can result in reaction at this center.

(b) When the functional group used for linking

35   to the targeting director is reactive in

the synthetic steps involved in the dendrimer synthesis, it can be protected by use of a protecting group, which renders the group unreactive to the synthetic procedures involved, but can itself be readily removed in a manner which does not alter the integrity of the remainder of the macromolecule.

(c)   In the event that no simple protecting group can be found for the reactive functionality to be used for linking with the targeting director, a synthetic precursor can be used which is unreactive in all the synthetic procedures used in the dendrimer synthesis. On completion of the synthesis, this functional group must be readily convertible into the desired linking group in a manner which does not alter the integrity of the remainder of the molecule.

(d)   Coupling (covalently) the desired reactive functional group onto a preformed dendrimer. The reagent used must contain a functionality which is readily reacted with the terminal functional groups of the dendrimer. The functional group to be ultimately used to link with the targeting agent can be in its final form, as a protected functionality, or as a synthetic precursor. The form in which this linking functionality is used depends on its integrity during the synthetic procedure to be utilized, and the ability of the
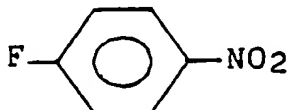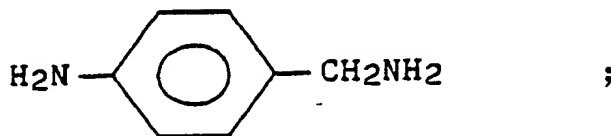
final macromolecule to withstand any
conditions necessary to make this group
available for linking.

5

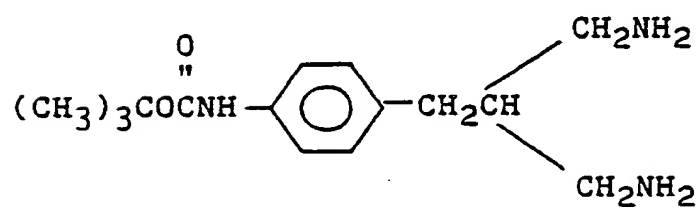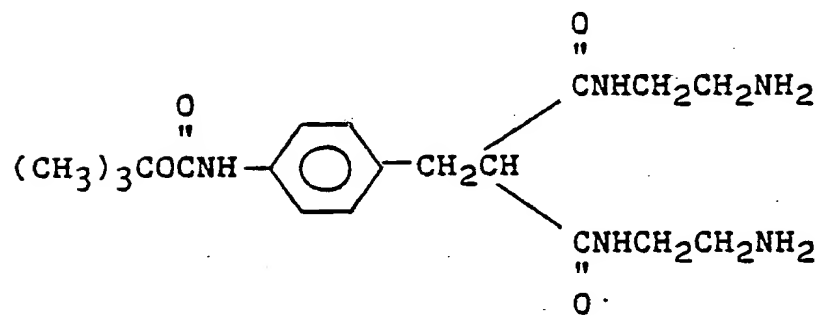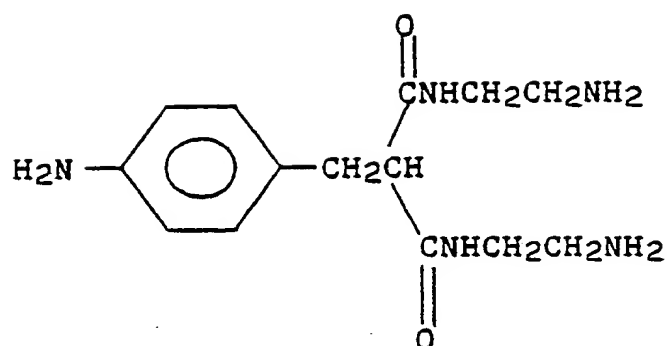For example, the preferred route for PEI uses

$$F - \langle \bigcirc \rangle - NO_2$$

10

Examples of heterofunctional initiators for use
in (1) above, include the following illustrative
examples:

15

$$H_2N - \langle \bigcirc \rangle - CH_2NH_2 \quad ;$$

20

25

30

35

$$H_2N \underset{}{\bigcirc} CH_2CH \overset{\overset{O}{\parallel}}{\underset{\underset{O}{\parallel}}{\overset{CNHCH_2CH_2NH_2}{\underset{CNHCH_2CH_2NH_2}{}}}}$$;

$$(CH_3)_3COCNH \underset{}{\bigcirc} CH_2CH \overset{\overset{O}{\parallel}}{\underset{\underset{O}{\parallel}}{\overset{CNHCH_2CH_2NH_2}{\underset{CNHCH_2CH_2NH_2}{}}}}$$;

$$(CH_3)_3COCNH \underset{}{\bigcirc} CH_2CH \overset{CH_2NH_2}{\underset{CH_2NH_2}{}}$$;

30

35

5   H2N—⟨◯⟩—CH2CH(NH2)CH2NH2   ;

10

    H2N—⟨◯⟩—CH2CH2NH2   ;

15

20   H2N—⟨◯⟩—CH2CH(CH2NH2)CH2NH2   ;

25

    O2N—⟨◯⟩—CH2CH2NH2   ;

30

35

CH$_2$NH$_2$

5

O$_2$N —⬡— CH$_2$CH — CH$_2$NH$_2$; and

CH$_2$NH$_2$

1C

CH$_2$CH$_2$NH$_2$

CH$_2$NCH$_2$CH$_2$NH$_2$

1E O$_2$N —⬡— CH$_2$CH

CH$_2$NCH$_2$CH$_2$NH$_2$

CH$_2$CH$_2$NH$_2$
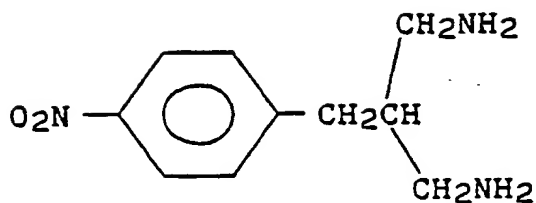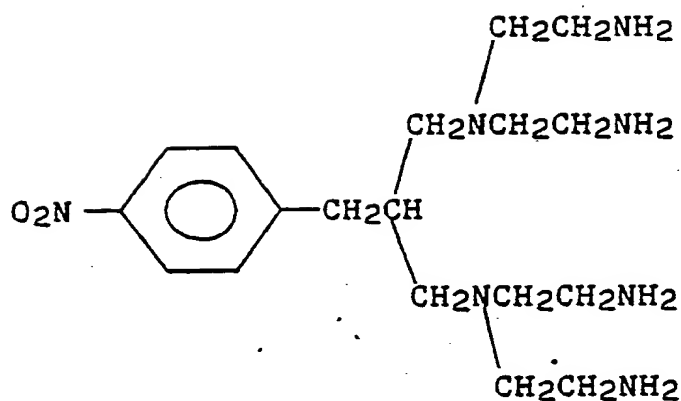
2C
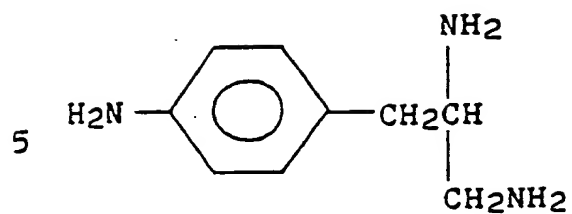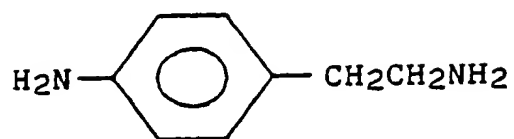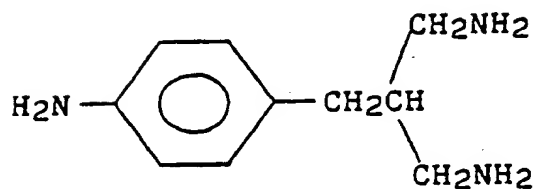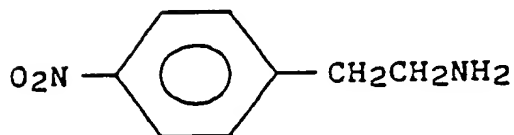
25   There are several chemistries of particular
importance:

1) Starburst Polyamidoamides ("PAMAM") Chemistry;

2) Starburst Polyethyleneimines ("PEI") Chemistry;

30   3) Starburst PEI compound with a surface of PAMAM;

4) Starburst polyether ("PE") chemistry.

Modifications of the dendrimer surface
functionalities may provide other useful functional

35

groups such as the following:

$-OPO_3H_2$, $-PO_3H_2$, $-PO_3H^{(-1)}$, $-PO_3^{(-2)}$, $-CO_2^{(-1)}$, $-SO_2H$, $-SO_2^{(-1)}$, $-SO_3H$, $-SO_3^{(-1)}$, $-NR^1R^2$, $-R^5$, $-OH$, $-OR^1$, $-NH_2$, polyethers, perfluorinated alkyl, $-\overset{\text{O}}{\underset{}{C}}NHR^1$, $-\overset{\text{O}}{\underset{}{C}}OH$,
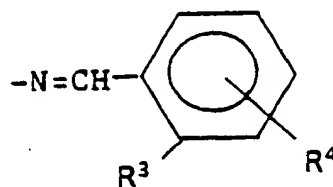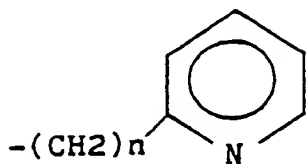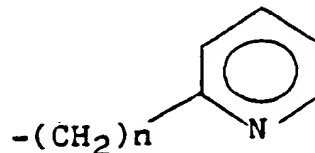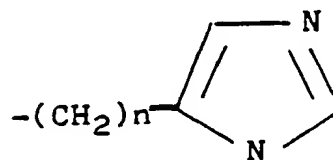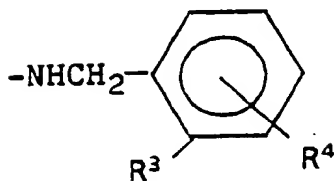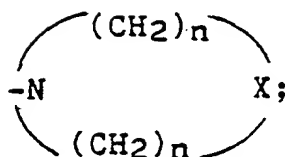
wherein
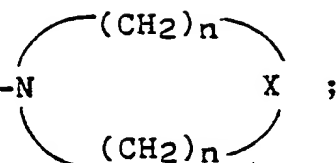
R represents alkyl, aryl or hydrogen;

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R¹ represents alkyl, aryl, hydrogen, or $-N\overset{\displaystyle (CH_2)_n}{\underset{\displaystyle (CH_2)_n}{\diagup\diagdown}}X$;

R² represents alkyl, aryl, or $-N\overset{\displaystyle (CH_2)_n}{\underset{\displaystyle (CH_2)_n}{\diagup\diagdown}}X$;

R³ represents -OH, -SH, -CO₂H, -SO₂H, or -SO₃H;

R⁴ represents alkyl, aryl, alkoxy, hydroxyl, mercapto, carboxyl, nitro, hydrogen, bromo, chloro, iodo, or fluoro;

R⁵ represents alkyl;

x represents NR, O or S; and

n represents the integer 1, 2 or 3.

The choice of functional group depends upon the particular end use for which the dendrimer is designed.

The following examples further illustrate the invention but are not to be construed as a limitation on the scope of the invention. The lettered examples concern the preparation of starting materials; the numbered examples concern the preparation of products.

Example A: Preparation of 2-Carboxamido-3-(4'-nitro-phenyl)-propanamide.

p-Nitrobenzyl malonate diethylester (2.4 grams (g), 8.13 mmole) was dissolved in 35 ml of methanol. The solution was heated to 50-55°C with stirring and a stream of anhydrous ammonia was bubbled through the solution for 64 hours. The solution was cooled and the

white, flocculant product was filtered and
recrystallized from 225 milliliters (ml) of boiling
methanol to afford 1.85 g (7.80 mmole) of bis amide in
96% yield (mp = 235.6°C(d)).

5

The structure was confirmed by MS,^{1}H and ^{13}C NMR
spectroscopy.

Anal: Calc. for $C_{10}H_{11}O_4N_3$

10

	C	H	N
Theo:	50.63	4.69	17.72
Found:	50.75	4.81	17.94

15 Example B: Preparation of 1-Amino-2-(aminomethyl)-3-
(4'-nitrophenyl)propane.

2-Carboxamido-3-(4'nitrophenyl)propanamide (2.0
g, 8.43 mmole) was slurried in 35 ml of dry tetrahydro-
furan under a nitrogen atmosphere with stirring. To
20 this mixture was added borane/tetrahydrofuran complex
(106 ml, 106 mmole) via syringe. The reaction mixture
was then heated to reflux for 48 hours during which
time the suspended amide dissolved. The solution was
cooled and the tetrahydrofuran was removed in vacuo
25 using a rotary evaporator. The crude product and
borane residue was dissolved in 50 ml of ethanol and
this solution was purged with anhydrous hydrogen
chloride gas. The solution was refluxed for 1 hour and
the solvent removed in vacuo. The crude hydrochloride
30 salt was dissolved in 15 ml of deionized water and
extracted with two 50 ml portions of methylene
chloride. The aqueous layer was cooled in an ice bath
under an argon blanket and 50% sodium hydroxide was
35 slowly added until basic pH=11.7. The basic aqueous
layer was extracted with four 25 ml portions of

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methylene chloride and these combined extracts were
evaporated (rotary) to give 1.45 g of amber colored
oil. This oil was triturated with diethyl ether (50
ml) and filtered under pressure through a short silica
5   gel (grade 62 Aldrich) column. The column was washed
with 100 ml of ether and the combined filtrates were
vacuum evaporated giving 1.05 g (5.02 mmole) of the
titled diamine as a clear oil (mp = 275-278°C(d) bis HCl
salt).

10   The structure was confirmed by MS, ^{1}H and ^{13}C NMR
spectroscopy.

<u>Anal:</u> Calc. for $C_{10}H_{17}N_3O_2Cl_2$

15

	C	H	N
Theo:	42.57	6.07	14.89
Found:	43.00	6.14	15.31

20   <u>Example C:</u> Preparation of 1-Amino-2-(aminomethyl)-3-
(4'-aminophenyl)propane.

Borane/tetrahydrofuran solution (70 ml, 70
mmole) was added under nitrogen <u>via</u> a cannula needle to
25   a flask containing 4-amino-benzyl malonamide (1.5 g,
7.24 mmole) with stirring. The solution was brought to
reflux for 40 hours. The colorless solution was cooled
and excess tetrahydrofuran was removed by rotary
evaporation leaving a clear gelatinous oil. Methanol
30   (50 ml) was cautiously added to the oil with notable
gas evolution. Dry hydrogen chloride was bubbled
through the suspension to effect dissolution and the
solution was then refluxed for 1 minute. The
methanol/HCl was rotary evaporated and the resulting
35   hydrochloride salt was carried through the same
dissolution/reflux procedure again. The hydrochloride

salt obtained was dissolved in 10 ml of water and cooled in an ice bath under argon. Concentrated sodium hydroxide (50%) was added slowly with stirring to pH=11. The aqueous portion was then extracted with 2 X

5 100 ml portions of chloroform which were combined and filtered through a short silica gel plug without drying. The solvent was removed _in_ _vacuo_ (rotary) affording the title compound (0.90 g, 5.02 mmole) in 70% yield (Rf=0.65 - CHCl3/MeOH/NH4OH conc - 2/2/1).

10 The structure was confirmed by ^{1}H and ^{13}C NMR and used without further purification.

Example D: Preparation of 6-(4-Aminobenzyl)-1,4,8,11-
tetraaza-5,7-dioxoundecane.

15

4-Aminobenzyl malonate dimethylester (2.03 g, 8.43 mmole) was dissolved in 10 ml of methanol. This solution was added dropwise to a stirred solution of freshly distilled ethylene diamine (6.00 g, 103.4

20 mmole) in 10 ml of methanol under nitrogen over a 2 hour period. The clear solution was stirred for 4 days and Thin Layer Chromotography (TLC) analysis indicated total conversion of diester (Rf = 0.91) to the bis amide (Rf = 0.42 - 20% conc NH4OH/80% ethanol). This

25 material was strongly ninhydrin positive. The methanol and excess diamine were removed on a rotary evaporator and the resulting white solid was vacuum dried (10^{-1} mm, 50°C) overnight to afford crude product (2.45g, 8.36

30 mmole) in 99% yield. An analytical sample was recrystallized from chloroform/hexane, MP = 160-161°C. The mass spectral, ^{1}H and ^{13}C NMR data were consistent with the proposed structure.

35

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Example E: Reaction of Mesyl Aziridine with 1-Amino-2-(aminomethyl)-3-(4-nitrophenyl)propane.

1-Amino-2-(aminomethyl)-3-(4-nitrophenyl)-
propane (400 mg, 1.91 mmole, >96% pure) was dissolved
5   in 10.5 ml of absolute ethanol under nitrogen. Mesyl
aziridine (950 mg, 7.85 mmole) was added to the stirred
diamine solution as a solid. The reaction was stirred
at 25°C for 14 hours using a magnetic stirrer and during
this period a white, gummy residue formed on the sides
10   of the flask. The ethanol was decanted and the residue
was triturated with another 15 ml portion of ethanol to
remove any unreacted aziridine. The gummy product was
vacuum dried (10^{1}mm, 25°C) to afford the tetrakis methyl
sulfonamide (1.0 g, 1.44 mmole) in 75% yield (R$_f$ = 0.74
15   - NH4OH/ethanol - 20/80). The structure was confirmed
by ^{1}H and ^{13}C nuclear magnetic resonance (NMR)
spectroscopy.

20   Example F: Preparation of 2-(4-Nitrobenzyl)-1,3-(bis-
N,N-2-aminoethyl)diaminopropane.

The crude methylsulfonamide (650 mg, 0.94
mmole) was dissolved in 5 ml of nitrogen purged,
concentrated sulfuric acid (98%). This solution was
25   maintained under nitrogen and heated to 143-146°C for 27
minutes with vigorous stirring. A slight darkening was
noted and the cooled solution was poured into a stirred
solution of ether (60 ml). The precipitated white salt
30   cake was filtered and immediately dissolved in 10 ml of
deionized water. The pH of the solution was adjusted
to pH=11 with 50% NaOH under argon with cooling. The
resulting solution was mixed with 90 ml of ethanol and
the precipitated inorganic salts were filtered. The
35   solvent was removed from the crude amine under reduced
pressure and to the resulting light brown oil was added

190 ml of toluene under nitrogen. The mixture was
stirred vigorously and water was removed through
azeotropic distillation (Dean-Stark trap) until the
remaining toluene acquired a light yellow color (30-40
5 ml remaining in pot). The toluene was cooled and
decanted from the dark, intractable residues and salt.
This solution was stripped of solvent _in vacuo_ and the
resulting light yellow oil was vacuum dried (0.2 mm,
35°C) overnight affording 210 mg of the product (60%)
10 which was characterized by MS, ^1H and ^{13}C NMR.

Example G: Preparation of a starburst polymer
(containing an aniline derivative) of one half
generation represented by the following scheme:

15

$$H_2N - \langle\bigcirc\rangle - CH_2CH(\overset{O}{\overset{\|}{C}}NHCH_2CH_2NH_2)_2 \quad + \quad \xrightarrow[CH_3OH]{H_2C=CHCOCH_3 \overset{O}{\overset{\|}{}}}$$

20

Compound #1

25

$$H_2N - \langle\bigcirc\rangle - CH_2CH(\overset{O}{\overset{\|}{C}}NHCH_2CH_2N(CH_2CH_2\overset{O}{\overset{\|}{C}}OCH_3)_2)_2$$
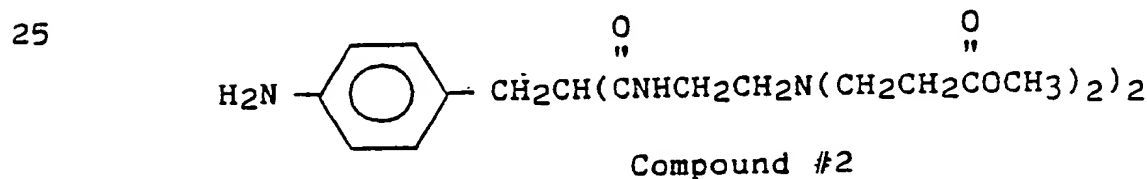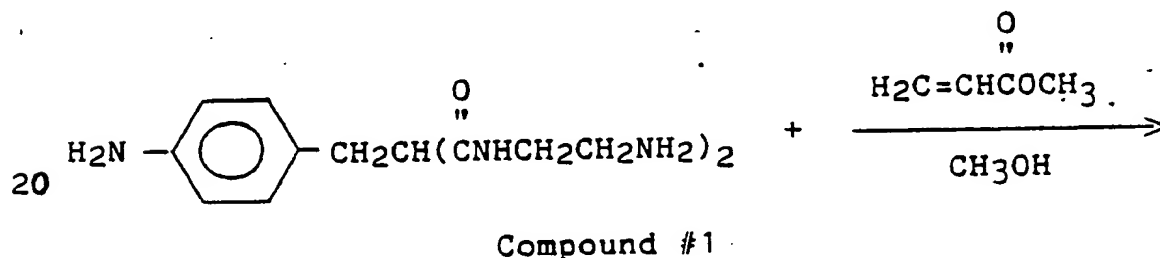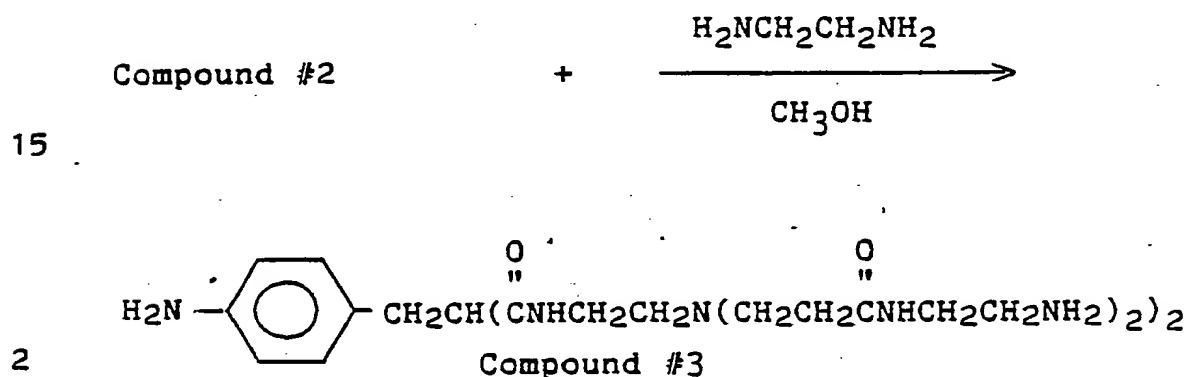
Compound #2

30   Methyl acrylate (2.09 g, 24 mmole) was
dissolved in methanol (15 ml). The compound 6-(4-
aminobenzyl)-1,4,8,11-tetraaza-5,7-dioxoundecane (1.1
g, 3.8 mmole) (i.e., Compound #1) was dissolved in
methanol (10 ml) and was added slowly over 2 hours with
35 rigorous stirring to the methyl acrylate solution. The

-44-

reaction mixture was stirred for 48 hours at ambient
temperatures. The solvent was removed on the rotary
evaporator maintaining the temperature below 40°C. The
ester (Compound #2) was obtained as a yellow oil (2.6
5   g). No carboxyethylation of the aniline function was
observed.

Example H: Preparation of a starburst polymer
(containing an aniline moiety) of one generation;
10   represented by the following scheme:

Compound #2   + $\dfrac{H_2NCH_2CH_2NH_2}{CH_3OH}$ $\longrightarrow$

15

$$H_2N-\bigcirc-CH_2CH(\overset{\overset{O}{\|}}{C}NHCH_2CH_2N(CH_2CH_2\overset{\overset{O}{\|}}{C}NHCH_2CH_2NH_2)_2)_2$$

2   Compound #3

The ester (Compound #2) (2.6 g, 3.7 mmole) was
dissolved in methanol (100 ml). this was carefully
added to a vigorously stirring solution of ethylene
25   diamine (250 g, 4.18 mole) and methanol (100 ml) at
such a rate that the temperature did not rise above
40°C. After complete addition the reaction mixture was
stirred for 28 hours at 35-40°C (heating mantle). After
30   28 hours no ester groups were detectable by infrared
spectroscopy. The solvent was removed on the rotary
evaporator at 60°C. The excess ethylene diamine was
removed using a ternary azeotrope of toluene-methanol-
ethylene diamine. Finally all remaining toluene was
35   azeotroped with methanol. Removal of all the methanol

yielded 3.01 g of the product (Compound #3) as an
orange glassy solid.

Example I: Preparation of a starburst polymer
(containing an aniline moiety) of one and one half
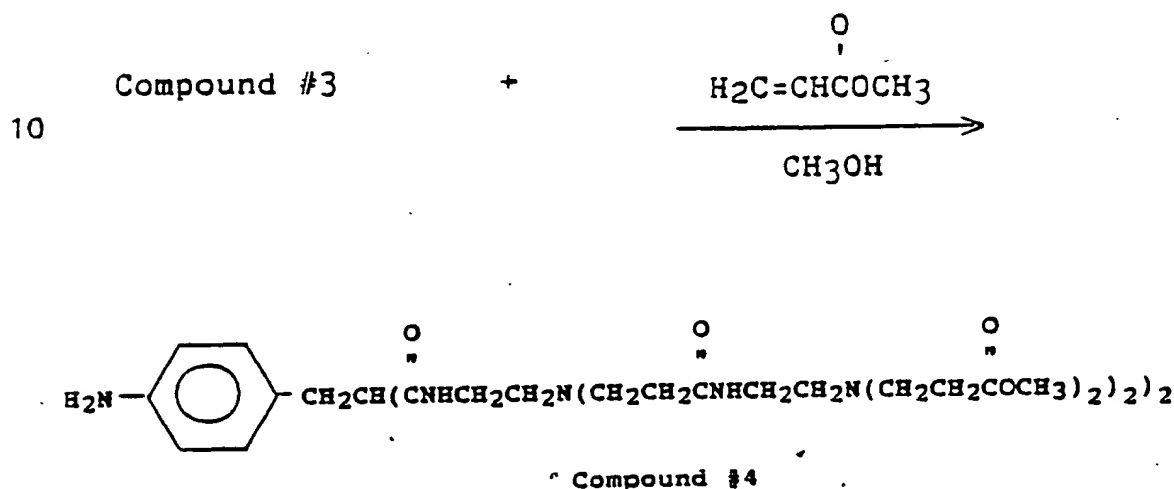generations represented by the following scheme:

$$Compound\ \#3\quad +\quad H_2C=CHCOCH_3 \xrightarrow{\quad CH_3OH\quad}$$

$$H_2N-\langle\bigcirc\rangle-CH_2CH(CNHCH_2CH_2N(CH_2CH_2CNHCH_2CH_2N(CH_2CH_2COCH_3)_2)_2)_2$$

Compound #4

The amine (Compound #3) (2.7 g, 3.6 mmole) was
dissolved in methanol (7 ml) and was added slowly over
one hour to a stirred solution of methyl acrylate (3.8
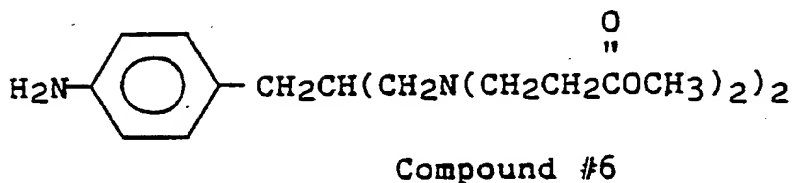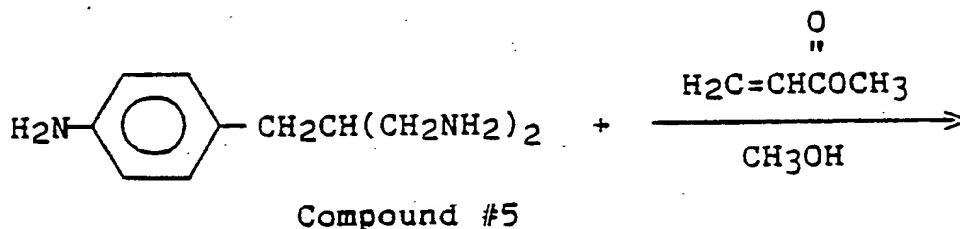g, 44 mmole) in methanol (15 ml) at ambient
temperatures. A slight warming of the solution was
observed during the addition. The solution was allowed
to stir at ambient temperatures for 16 hours. The
solvent was removed on the rotary evaporator at 40°C.
After removal of all the solvent and excess methyl
acrylate the ester (Compound #4) was obtained in: 4.7 g
yield as an orange oil.

<u>Example J:</u> Preparation of a starburst polymer
(containing an aniline moiety) of one half generation
represented by the following scheme:

5

$$H_2N-\langle\bigcirc\rangle-CH_2CH(CH_2NH_2)_2 \quad + \quad \xrightarrow[CH_3OH]{\overset{\overset{O}{\parallel}}{H_2C=CHCOCH_3}}$$

Compound #5

10

$$H_2N-\langle\bigcirc\rangle-CH_2CH(CH_2N(CH_2CH_2\overset{\overset{O}{\parallel}}{C}OCH_3)_2)_2$$

15

Compound #6

The triamine (Compound #5, the preparation of
this compound is shown in Example C) (0.42 g, 2.3

20 mmole) was dissolved in methanol (10 ml) and was added
dropwise over one hour to methyl acrylate (1.98 g, 23
mmole) in methanol (10 ml). The mixture was allowed to
stir at ambient temperatures for 48 hours. The solvent
was removed on the rotary evaporator, maintaining the

25 temperature at no higher than 40°C. The excess methyl
acrylate was removed by repeated azeotroping with
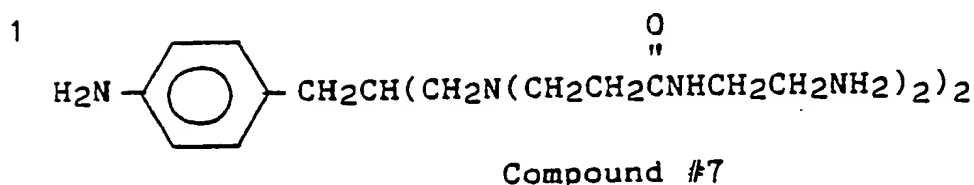methanol. The ester (Compound #6) was isolated as an
orange oil (1.24 g).

30

35

Example K: Preparation of a starburst polymer (containing an aniline moiety) of one generation; represented by the following scheme:

5

$$\text{Compound \#6} \quad + \quad \frac{\text{H}_2\text{NCH}_2\text{CH}_2\text{NH}_2}{\text{CH}_3\text{OH}} \quad \longrightarrow$$

1

$$\text{H}_2\text{N}-\bigcirc-\text{CH}_2\text{CH}(\text{CH}_2\text{N}(\text{CH}_2\text{CH}_2\overset{\overset{\displaystyle O}{\|}}{\text{C}}\text{NHCH}_2\text{CH}_2\text{NH}_2)_2)_2$$
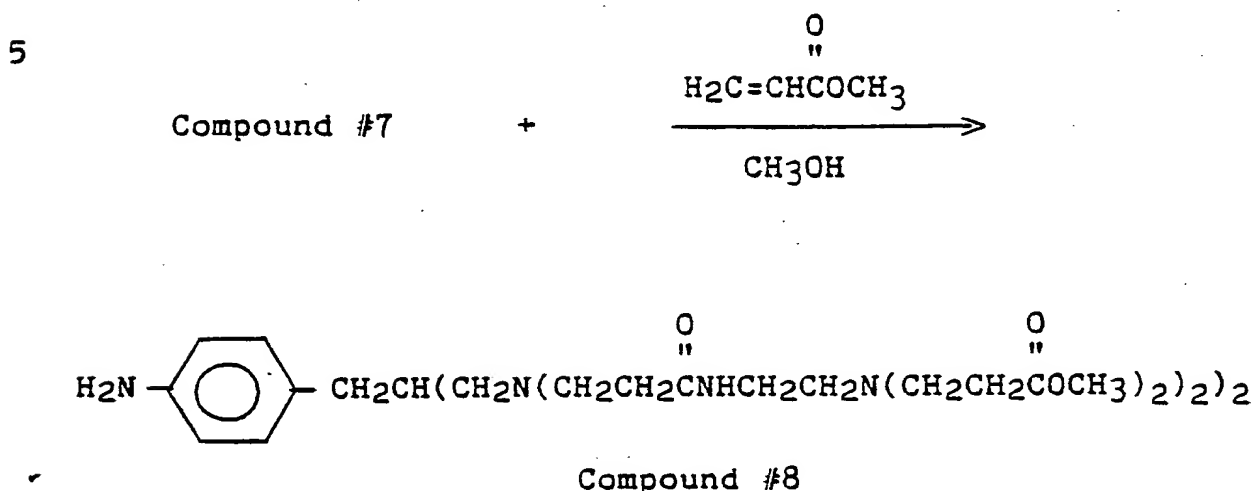
Compound #7

1

The ester (Compound #6) (1.24 g, 2.3 mmole) was dissolved in methanol (50 ml) and was added dropwise over two hours to ethylenediamine (73.4 g, 1.22 mole) in methanol (100 ml). A small exotherm was noted,

20 vigorous stirring was maintained. The solution was left to stir at ambient temperatures for 72 hours. The solvent was removed on the rotary evaporator at 60°C. The excess ethylene diamine was removed using a ternary azeotrope of toluene-methanol-ethylenediamine. Finally

25 all remaining toluene was removed with methanol and then pumping down with a vacuum pump for 48 hours gave the amine (Compound #7) (1.86 g) as a yellow/orange oil.

30

35

Example L: Preparation of a starburst polymer
(containing an aniline moiety) of one and one half
generations; represent by the following scheme:

5

$$
\text{Compound \#7} \quad + \quad \frac{H_2C{=}CHCOCH_3}{CH_3OH} \longrightarrow
$$

$H_2N{-}\langle\bigcirc\rangle{-}CH_2CH(CH_2N(CH_2CH_2CNHCH_2CH_2N(CH_2CH_2COCH_3)_2)_2)_2$

Compound #8

The amine (Compound #7) (1.45 g, trace of
methanol remained) was dissolved in methanol (100 ml)
and was added slowly over 1½ hours to a stirred
20   solution of methyl acrylate (5.80 g) in methanol (20
ml). The solution was allowed to stir for 24 hours at
room temperature. Removal of the solvent followed by
repeated azeotroping with methanol enabled the removal
25   of all the excess methyl acrylate. After pumping down
on a vacuum pump for 48 hours the ester (Compound #8)
was isolated as an orange oil (2.50 g, 1.8 mmole).

Example M: Hydrolysis of 4.5 generation dendrimer and
30   preparation of calcium salt.

4.5 Generation PAMAM (ester terminated,
initiated off NH3) (2.11 g, 10.92 meq) was dissolved in
25 ml of methanol and to it was added 10% NaOH (4.37
35   ml, 10.92 meq) (pH = 11.5-12). After 24 hours at room
temperature, the pH was about 9.5. After an additional

20 hours, the solution was rotovaped, 50 ml of toluene added, and rotovaped again.

The resulting oil was dissolved in 25 ml of methanol and precipitated as a white gum upon addition of 75 ml of diethyl ether. The liquid was decanted and the gum was rotovaped to give a very fine off-white powder which upon drying gives 2.16 g of product (98% yield). No ester groups were found upon NMR and infrared analysis.

The sodium salt of 4.5 Generation PAMAM (ester terminated, initiated from NH_3) was replaced by the calcium salt via dialysis. The sodium salt (1.03 g) was dissolved in 100 ml of water and passed through hollow fiber dialysis tubing (cut off = 5000) at 3 ml/minute. The exterior of the tubing was bathed in 5% $CaCl_2$ solution. This procedure was then repeated.

The resulting solution was again dialyzed, this time against water, then repeated two additional times.

Evaporation provided 0.6 g of wet solid, which was taken up in methanol (not totally soluble) and is dried to give 0.45 g of off-white crystals.

$C_{369}H_{592}O_{141}N_{91}Ca_{24}$ Calc. - 10.10% Ca^{++}

M Wt. = 9526.3 Calc. = C-4432.1, H-601.8, O-2255.9,
N-1274.6, Ca-961.9)

Theo: C-46.5, H-6.32, N-13.38, Ca-10.10
Found: C-47.34, H-7.00, N-13.55, Ca-8.83

Example N: Preparation of dendrimers with terminal
carboxylate groups.

Half-generation starburst polyamidoamines were
hydrolyzed to convert their terminal methyl ester
5   groups to carboxylates. This generated spheroidal
molecules with negative charges dispersed on the
periphery. The dendrimers hydrolyzed ranged from 0.5
generation (three carboxylates) to 6.5 generation (192
carboxylates).
10

The products could be generated as Na^+, K^+, Cs^+
or Rb^+ salts.

Example O: N-t-butoxycarbonyl-4-aminobenzyl malonate
15   dimethylester

4-Aminobenzyl malonate dimethylester (11.62 g,
49 mmol) was dissolved in 50 ml of t-butanol:water
20   (60:40 with stirring. Di-t-butoxydicarbonate (19.79 g,
90 mmol) was added and the reaction mixture stirred
overnight. The butanol was removed on the rotary
evaporator, resulting in a yellow suspension of the
25   product in water. Extraction into methylene chloride,
drying ($MgSO_4$) and evaporation gave a yellow oil (21.05
g, contaminated by di-t-butoxydicarbonate).
Recrystallization from 2-propanol:water (75:25) yield
pale yellow crystals (11.1 g, 33 mmol, 67%). The
30   structure was confirmed by 13_C NMR and purity checked
by hplc analysis (spherisorb ODS-1, 0.05M H_3PO_4 pH 3:
CH_3CN 55:45). The material was used without further
purification.

35

Example P: N-t-butoxycarbonyl-6-(4-aminobenzyl)-
1,4,8,11-tetraaza-5,7-dioxoundecane

N-t-butoxycarbonyl-4-aminobenzyl malonate
dimethylester (8.82 g 26 mmol), prepared in Example O,
5   was dissolved in 50 ml of methanol, This solution was
added dropwise (2 hours) to a solution of freshly
distilled ethylenediamine (188 g 3.13 mole) and 20 ml
of methanol, under a nitrogen atmosphere. The solution
was allowed to stir for 24 hours. The ethylene
10   diamine/methanol solution was removed on the rotary
evaporator. The product was dissolved in methanol and
toluene added. Solvent removal on the rotary
evaporator gave the crude product as a white solid
(10.70 g contaminated with ethylenediamine). The
15   sample was divided into two samples for purification.
Azeotropic removal of ethylenediamine with toluene,
using a soxhlet extractor with sulphonated ion exchange
beads in the thimble to trap the ethylenediamine,
20   resulted in partial decomposition of the product,
giving a brown oil. The remaining product was isolated
as a white solid from the toluene on cooling (2.3 g
approximately 50 percent). Analysis of a 10 percent
solution in methanol by gas chromatography (Column,
25   Tenax 60/80) showed no ethylenediamine detectable in
the sample (<0.1 percent). The second fraction was
dissolved in methanol to give a 10 percent solution (by
weight) and purified from the ethylenediamine by
30   reverse osmosis, using methanol as the solvent. (The
membrane used was a Filmtec FT-30 , in an Amicon TC1R
thin channel separator, the ethylenediamine crossing
the membrane.) The product was isolated as a white
solid (2.7 g), in which no detectable amounts of
35   ethylenediamine could be found by gas chromatography.
The ^{13}C NMR data and HLPC analysis (Spherisorb ODS-1,

0.05M H_3PO_4 pH 3:CH_3CN 55:45) were consistent with the proposed structure. The product was used with no further purification.

5   Example Q: Preparation of a starburst dendrimer of one half generation from N-t-butoxycarbonyl-6-(4-aminobenzyl)-1,4,8,11-tetraaza-5,7-dioxoundecane

N-t-butoxycarbonyl-6-(4-aminobenzyl)-1,4,8,11-tetraaza-5,7-dioxoundecane (5.0 g 13 mmol), prepared in
10 Example P, was dissolved in 100 ml of methanol. Methyl acrylate (6.12 g, 68 mmol) was added and the solution stirred at ambient temperatures for 72 hours. The reaction was monitored by HPLC (Spherisorb ODS1, Acetonitrile: 0.04M Ammonium acetate 40:60) to optimize
15 conversion to the desired product. The solution was concentrated to 30 percent solids, and methyl acrylate (3.0 g 32 mmol) was added. The reaction mixture was stirred at ambient temperatures until no partially
20 alkylated products were detectable by HPLC (24 hours). Removal of the solvent at 30°C by rotary evaporation, and pumping down at 1 mm Hg for 24 hours gave the product as yellow viscous oil, yield 7.81 g. The 13C NMR data was consistent with the proposed structure.
25 The product was used without further purification.

Example R: Preparation of a starburst dendrimer of one full generation from N-t-butoxycarbonyl-6-(4-aminobenzyl)-1,4,8,11-tetraaza-5,7-dioxoundecane
30
The half generation product (Example Q) (7.70 g, 10.45 mmol) was dissolved in 75 ml of methanol and added dropwise over 2 hours to a stirred solution of ethylenediamine (400 ml, 7.41 mol) and methanol (50
35 ml). The reaction mixture was stirred at ambient temperatures for 48 hours. The ethylenediamine and

methanol were removed by rotary evaporation to give a
yellow oil (11.8 g contaminated with ethylene diamine).
The product was dissolved in 90 ml of methanol, and
purified from the ethylenediamine by reverse osmosis

5   (Filmtec FT-30 membrane and Amicon TC1R thin channel
separator, methanol as solvent). After 48 hours, no
ethylenediamine could be detected by gas chromatography
(Column, Tenax 60/80). Removal of the solvent on the
rotary evaporator, followed by pumping down on a vacuum

10 line for 24 hours gave the product as a yellow glassy
solid (6.72 g). Analysis by HPLC, PLRP-S column,
acetonitrile:0.015M NaOH, 10-20 percent gradient in 20
min.) and 13C NMR analysis was consistent with the
proposed structure.

15

Example S: Preparation of a starburst polymer of one
and one half generation from N-t-butoxycarbonyl-6-(4-
aminobenzyl)-1,4,8,11-tetraaza-5,7-dioxoundecane

20   The one generation product (Example R) (2.14 g,
25 mmol) was dissolved in 12.5 ml of methanol, and
methyl acrylate (3.5 g, 39 mmol) in 5 ml of methanol
was added. The solution was stirred at ambient
temperatures for 48 hours, monitoring the progress of

25 the reaction by HPLC (Spherisorb ODS-1, acetonitrile:
0.04M ammonium acetate, 60:40). A second aliquot of
methyl acrylate was added (3.5 g 39 mmol) and the
reaction mixture stirred at ambient temperatures for a
further 72 hours. Removal of the solvent on the rotary

30 evaporator gave the product as a yellow oil (3.9 g)
after pumping down overnight with a vacuum pump. The
product was used with no further purification.

35

Example T: Preparation of a starburst polymer of two
full generations from N-t-butoxycarbonyl-6-(4-
aminobenzyl)-1,4,8,11-tetraaza-5,7-dioxoundecane

The one and one half generation product

5   (Example S) (3.9 g, 2.5 mmol) was dissolved in 50 ml of
methanol, and was added dropwise over 2 hours to a
stirred solution of ethylenediamine (600 g, 10 mol) and
methanol (50 ml). The solution was stirred at ambient
temperatures under an atmosphere of nitrogen for 96

10   hours. The ethylenediamine/methanol was removed on the
rotary evaporator to give a yellow glassy solid (4.4 g
contaminated with ethylenediamine). A 10 percent
solution of the product was made in methanol, and
purified from the ethylenediamine by reverse osmosis

15   (membrane used as a Filmtec FT-30, in an Amicon TC1R
thin channel separator) until no ethylenediamine could
be detected by gas chromatography (Column, Tenax 60/80.
Removal of the solvent gave the product as a yellow
glassy solid (3.52 g). The 13C NMR data and HPLC

20   analysis (PLRP-S column, acetonitrile:0.015 M NaOH, 10
to 20 percent gradient in 20 minutes, were consistent
with the proposed structure.

25   Example U: Reaction of the two generation starburst
with Bromoacetic Acid to give a methylene carboxylate
terminated starburst dendrimer

The second generation product (Example T) (0.22
g, 0.13 mmol) was dissolved in 15 ml of deionized water

30   and the temperature equilibrated at 40.5°C. Bromoacetic
acid (0.48 g, 3.5 mmol) and lithium hydroxide (0.13 g,
3.3 mmol) were dissolved in 5 ml of deionized water,
and added to the reaction mixture. The reaction pH was

35   carefully maintained at 9, with the use of a pH stat
(titrating with 0.1N NaOH), at 40.5°C overnight.

Monitoring by reverse phase HPLC, (Spherisorb ODS-1
column, eluent 0.25 M H_3PO_4 pH 3 [NaOH]; acetonitrile
85:15) confirmed the synthesis of predominantly a
single component.

5

Example V: Preparation of Isothiocyanato functionalized
second generation methylene-carboxylate terminated
starburst dendrimer

        Five ml of a 2.8 mM solution of the second
10. generation methylenecarboxylate terminated starburst
dendrimer (Example U) was diluted with 20 ml water and
the pH adjusted to 0.5 with concentrated hydrochloric
acid. After one hour at room temperature the mixture
was analyzed by HPLC to verify the removal of the
15 butoxycarbonyl group and then treated with 50 percent
sodium hydroxide to being the pH to 7. A pH stat
(titrating with 0.1 N NaOH) was used to maintain the pH
at 7 and 225 µl thiophosgene was added.· After 15
20 minutes at room temperature the pH of the mixture was
adjusted to 5 with 1N HCl. The mixture washed with
chloroform (20 ml x 2) then concentrated on a rotary
evaporator at reduced pressure. The residue recovered
0.91 g is a mixture of the isothiocyanate and salts.
25.

Example W: Preparation of second generation starburst
polyethyleneimine-methane sulfonamide

        To a solution of 125 g N-methanesulfonyl-
30 aziridine in 50 ml ethanol was added 25.0 g tris(2-
aminoethyl)amine. The solution was stirred at room
temperature for 4 days. Water was added to the
reaction mixture as needed to maintain the homogeneity
of the solution. The solvent was removed by
35 distillation in vacuo to give the 2nd generation

starburst PEI-methane sulfonamide as a yellow glass
(161 g).

Example X: Cleavage of methane sulfonamides to form
5   second generation starburst polyethyleneimine

        A solution of 5.0 g of second generation
starburst PEI-methane sulfonamide, from Example W in 20
ml of 38 percent HCL was sealed in a glass ampoule.
10 The ampoule was heated at 160°C for 16 hours, then
cooled in an ice bath and opened. The solvent was
removed by distillation in vacuo and the residue
dissolved in water. After adjusting the pH of the
solution to greater than or equal to 10 with 50 percent
15 NaOH, the solvent was removed by distillation in vacuo.
Toluene (150 ml) was added to the residue and the
mixture heated at reflux under a Dean-Stark trap until
no more water could be removed. The solution was
filtered to remove salts and the filtrate concentrated
20 in vacuo to give 1.9 g second generation starburst PEI
as a yellow oil.

Example Y: Preparation of third generation starburst
polyethyleneimine-methane sulfonamide
25

        To a solution of 10.1 g second generation
starburst PEI, from Example X, in 100 ml ethanol was
added 36.6 g N-methanesulfonylaziridine. The solution
was stirred at room temperature for 1 week. Water was
30 added as needed to maintain the homogeneity of the
solution. The solvent was removed by distillation in
vacuo to give third generation starburst PEI-methane
sulfonamide as a yellow glass (45.3 g).

35

Example Z: Cleavage of methane sulfonamides to form 3rd gen starburst polyethyleneimine

5   The methane sulfonamide groups of third generation starburst PEI-methane sulfonamide (5.0 g), from Example Y, were removed by the same procedure as described for the second generation material in Example X to give 2.3 g third generation starburst PEI as a
10   yellow oil.

Example AA: Preparation of a methylenecarboxylate terminated second generation starburst polyamidoamine (initiated from ammonia)

15   The second generation starburst polyamidoamine (2.71 g, 2.6 mmol) and bromoacetic acid (4.39 g, 31.6 mmol) were dissolved in 30 ml of deionized water and the pH adjusted to 9.7 with 5N NaOH using a pH stat. The reaction was maintained at this pH for a half hour,
20   and the temperature was slowly raised to 60°C and was maintained at 60°C for three hours at constant pH. The pH was raised to 10.3, and the reaction mixture remained under control of the pH stat at ambient temperatures overnight. The reaction mixture was
25   refluxed for a further four hours prior to work up. Removal of the solvent, and azeotroping the final traces of water with methanol gave the product as a pale yellow powder (8.7 g, contaminated with sodium
30   bromide). The ^{13}C NMR spectrum was consistent with the propose structure (with some contamination due to a small amount of defected material as a result of some monoalkylation).

35

<u>Example BB</u>: Preparation of a methylenecarboxylate
terminated second generation starburst
polyethyleneimine (initiated from ammonia)

5   The second generation starburst
polyethyleneimine (2.73 g, 6.7 mmol), from Example X,
and bromoacetic acid (11.29 g 81 mmol) were dissolved
in 30 ml of deionized water. The pH was slowly raised
to pH 9.5 maintaining the temperature below 30°C. The
temperature was raised slowly to 55°C, and the reaction
10   pH maintained at 9.5 for 6 hours with the aid of a pH
stat (titrating with 5N NaOH). The pH was raised to
10.2, and maintained at that pH overnight. Removal of
the solvent on the rotary evaporator, and azeotroping
the final traces of water using methanol, gave the
15   product as a yellow powder (17.9 g, contaminated with
sodium bromide). The ^{13}C NMR spectrum was consistent
with the proposed structure (with some contamination
due to a small amount of defected material as a result
20   of some monoalkylation).

<u>Example CC</u>: Preparation of a 3.5, 4.5, 4.4 and 6.5
generation starburst PAMAM:

25   To a 10 weight percent methanolic solution of
2.46 g 3 generation PAMAM starburst was added 2.32 g of
methyl acrylate. This mixture was allowed to sit at
room temperature of 64 hours. After solvent and excess
methyl acrylate removal, 4.82 g of product was
30   recovered (105 percent of theoretical).

      Preparation of higher 1/2 generation starburst
PAMAM's:

35   Generations 4.5, 5.5 and 6.5 were prepared as
described above with no significant differences in

reactant concentrations, reactant mole ratios or reaction times.

Example DD: Preparation of 4, 5 and 6 generation starburst PAMAM:

To 2000 g of predistilled ethylenediamine was added 5.4 g of 4.5 generation starburst PAMAM as a 15 weight percent solution in methanol. This was allowed to sit at room temperature for 48 hours. The methanol and most of the excess ethylenediamine were removed by rotary evaporation under water aspirator vacuum at temperature less than 60°C. The total weight of product recovered was 8.07g. Gas chromatography indicated that the product still contained 34 weight percent ethylene-diamine at this point. A 5.94 g portion of this product was dissolved in 100 ml methanol and ultrafiltered to remove the residual ethylenediamine. The filtration was run using an Amicon TC1R thin channel recirculating separator equipped with an Amicon YM2 membrane. An in-line pressure relief valve was used to maintain 55 psig (380 kPa) pressure across the membrane. The 100 ml was first concentrated to 15 ml by forcing solvent flow exclusively through the membrane. After this initial concentration, the flow was converted to a constant volume retentate recycle mode for 18 hours. After this time, 60 ml of methanol was passed over the membrane to recover product still in the module and associated tubing. The product was stripped of solvent and 2.53 g of 5 generation starburst PAMAM was recovered. Analysis by gas chromatography indicated 0.3 percent residual ethylene-diamine remained in the product.

Preparation of generation 4 and 6 proceeded as above with the only difference being the weight ratio of ethylenediamine to starting material. To prepare 4th generation this ratio was 200:1 and for 6th

5   generation this ratio was 730:1.

Example 1: Preparation of a product containing more than one rhodium atom per starburst polymer.

2.5 Generation PAMAM (ester terminated,

10   initiated from NH3) (0.18 g, 0.087 mmole) and RhCl3·3H2O (0.09 g, 0.3 mmole) were mixed in dimethylformamide (DMF) (15 ml) and heated for 4 hours at 70°C. The solution turned crimson and most of the rhodium was taken up. The unreacted rhodium was

15   removed by filtration and the solvent removed on the rotary evaporator. The oil formed was chloroform soluble. This was washed with water and dried (MgSO4) before removal of solvent to yield a red oil (0.18 g).

20   The NMR spectrum was recorded in CDCl3 only minor differences were noted between the chelated and unchelated starburst. Dilution of some of this CDCl3 solution with ethanol followed by NaBH4 addition resulted in rhodium precipitation. RhCl3·3H2O is

25   insoluble in chloroform and in chloroform starburst solution thus confirming chelation.

Example 2: Preparation of a product containing Pd chelated to starburst polymer.

30

3.5 Generation PAMAM (ester terminated, initiated from NH3) (1.1 g, 0.24 mmole) was dissolved with stirring into acetonitrile (50 ml). Palladium chloride (0.24 g, 1.4 mmole) was added and the solution

35   was heated at 70-75°C (water bath) overnight. The PdCl2 was taken up into the starburst. After removal of the

solvent, the NMR in CDCl3 confirmed that chelation had occurred. Dilution of the CDCl3 solution with ethanol and addition of NaBH4 resulted in precipitation of the palladium. The chelated product (1.23 g) was isolated

5 as a brown oil.

Example 3 : Attachment of herbicidal molecules (2,4-D) to the surface of starburst dendrimers.

10.   Third generation PAMAM (initiator core=NH3) (2.0 g, 0.8 mmole) was dissolved in H2O (10 ml) and combined with toluene (20 ml). The two-phase system was then stirred and cooled with an ice bath at which time the acid chloride of 2,4-D [2,4-dichlorophenoxy-

15 acetic acid] (2.4 g, 12 equiv) dissolved in toluene (10 ml) was added dropwise over 30 minutes. When the addition was nearly complete, NaOH (0.5 g, 12.5 mmole, 50% w/w solution) was added and the solution stirred for an additional two hours. The reaction mixture was

20 then evaporated to dryness and the resulting solid residue repeatedly taken up in CHCl3/MeOH (1:1) and filtered. The tan solid was not totally soluble in CHCl3 and appeared to be insoluble in water; however,

25. the addition of acetone facilitated dissolution. The tan solid was stirred in CHCl3 for 24 hours and the solution filtered (a sticky tan solid was obtained). After drying over MgSO4, the filtrate was concentrated to give a viscous orange oil which solidified on

30 standing. The ^{13}C NMR partial amidation at the surface by 2,4-D is consistent with the association of the 2,4-D to starburst dendrimer.

35

Example 4:   Incorporation of 2,4-dichlorophenoxyacetic
acid (2,4-D) into starburst dendrimers.

A widely accepted method for ascertaining
whether a "probe molecule" is included in the interior
5   of a micelle is to compare its carbon-13-spin lattice
relaxation times (T_1) in a non-micellized versus
micellized medium. A substantial decrease in T_1 for
the micellized medium is indicative of "probe molecule"
inclusion in the micelle. Since starburst dendrimers
10.   are "covalently fixed" analogs of micelles, this T_1
relaxation time technique was used to ascertain the
degree/extent to which various herbicide type molecules
were associated with starburst polyamidoamines. In the
following examples, T_1 values for 2,4-dichlorophenoxy-
15   acetic acid (I) (2,4-D) were determined in solvent
($CDCl_3$) and then compared to T_1 values in $CDCl_3$ at
various [I:dendrimer] molar ratios.

Inclusion of 2,4-D into various starburst polyamido-
20   amine dendrimers as a function of generation.

Various half generation (ester terminated,
initiated from NH_3) starburst polyamidoamine dendrimers
25   (Generation (Gen) = 0.5, 1.5, 2.5, 3.5, 4.5 and 5.5)
were combined with 2,4-dichlorophenoxyacetic acid (I)
in $CDCl_3$ to give an acid:tertiary amine ratio of 1:3.5
and molar ratios of acid:dendrimer of 1:86 as shown in
Table III. The relaxation times (T_1) obtained for the
30   various carbon atoms in 2,4-dichlorophenoxyacetic acid
and a generation = 3.5 starburst PAMAM dendrimers are
shown in Table IV, both for 1:1 acid/amine ratios and
for saturated solutions of 2,4-D.

35

Table III

Gen	(A) Acid/Amine	(B) Acid/Amine	(C) Acid/Total Nitrogen	(D) Molar Ratio (Acid/Starburst)
0.5	1	--	1	1
1.5	1	1.33	0.57	6
2.5	1 (3.5)*	1.11 (3.8)*	0.53 (1.8)*	9 (34)*
3.5	1 (3.0)*	1.05 (3.2)*	0.51 (1.6)*	20 (67)*
4.5	1	1.02	0.51	42
5.5	1	1.01	0.50	86

* represents examples of 2,4-D inclusion into the interior of the dendrimer in amounts greater than stoichiometric.

Table IV
T_1's for 2,4-D/$\overline{G} = 3.5$ PAMAM Starburst
Inclusion complex: Concentration Effects

Carbon	(A) 1:1 Acid/Amine		(B) Saturated with 2,4-D	
	T_1	^{13}C**	T_1	^{13}C**
1	3.19±.12	(152.73)	3.08±.09	(152.30)
3	0.34±.01	(128.64)	0.29±.01	(129.62)
5	0.38±.01	(127.41)	0.32±.01	(127.34)
2	3.28±.08	(125.79)	2.72±.68	(125.99)
4	4.58±.16	(123.27)	3.95±.07	(123.16)
6	0.31±.01	(114.66)	0.28±.01	(114.48)
CH_2	0.16±.01	(67.29)	0.146±.003	(66.79)
C=O	1.24±.07	(170.12)	-	-

** represents ^{13}C chemical shifts referenced to chloroform at 76.9 ppm..

These data show that larger than stoichiometric amounts
of 2,4-dichlorophenoxyacetic acid (i.e., [(I):Gen=3.5
dendrimer)] = 67 can be used without increasing the T_1
in any case in the saturated state (see Columns (A) and

5　　(B) in Table IV). In fact, the relaxation times T_1
(Column (B) are decreased slightly, thus indicating
that larger than stoichiometric amounts of 2,4-
dichlorophenoxyacetic acid can be included into the
interior of the dendrimer. For example, a molar ratio

10.　of

[(I):Gen=2.5 dendrimer]= 34 whereas [(I):Gen=3.5
dendrimer]= 67,

15　(see Column D in Table III).

Figure 3 is a plot of T_1 values for carbons-3,
5 and 6 in 2,4-dichlorophenoxyacetic acid as a function
of dendrimer generation (i.e., 0.5 → 5.5). A minimum

20　in T_1 is reached in all cases of generation 2.5 → 5.5,
thus indicating incorporation in that dendrimer
generation range is occurring. Figure 3 also includes
T_1 values for 2,4-D in the presence of triethylamine
[N(Et)$_3$] and N(Et)$_3$ + N-methylacetamide. It can be seen

25　that these values are much larger than for dendrimers G
= 1.5 → 5.5, thus further supporting molecular
incorporation into the dendrimer molecule.

Example 5: Demonstration of multiple chelation of

30　yttrium by a methylene carboxylate terminated second
generation starburst polyethyleneimine by trans
chelation from yttrium acetate

The starburst polyethyleneimine methylene
carboxylate terminated material (0.46 g 52. 5 percent

35　active, remainder sodium bromide, 0.18 mmol active
starburst dendrimer), from Example BB, was dissolved in

4.5 ml of deuterium oxide. The resultant pH was 11.5-12. A solution of yttrium acetate was prepared by dissolving yttrium chloride (0.15 g, 0.5 mmol) and sodium acetate (0.41 g, 0.5 mmol) in 1.5 ml of
5   deuterium oxide (2.9 moles of yttrium per mole of dendrimer). Aliquots of 0.5 ml of the yttrium acetate solution were added to the dendrimer solution and the ^{13}C NMR spectra recorded at 75.5 MHz.

10   The ^{13}C NMR spectrum of yttrium acetate shows two resonances, 184.7 ppm for the carboxyl carbon and 23.7 ppm for the methyl carbon, compared with 182.1 and 24.1 ppm for sodium acetate, and 177.7 and 20.7 ppm for
15   acetic acid (Sadtler ^{13}C NMR Standard Spectra). Monitoring the positions of these bands indicates degree of chelation with the starburst dendrimer. The most informative signal for the starburst dendrimer which is indicative of chelation is the α-CH$_2$ (of the
20   methylene carboxylate group involved in chelation), which appears at 58.4 ppm in the unchelated dendrimer, and 63.8 ppm in the chelated dendrimer. Upon chelation with yttrium, the spin lattice relaxation times of the time α-CH$_2$ shortens as expected from 0.24 ± 0.01s to
25   0.14 ± 0.01s, indicative of chelation.

       Following the addition of 0.5 ml of the yttrium acetate solution to the starburst dendrimer, all the
30   yttrium appeared to be chelated by the dendrimer, confirmed by the signals for the acetate being that of sodium acetate. The same observation was noted for the addition of a second 0.5 ml aliquot of the yttrium acetate solution. Upon addition of the third aliquot
35   of yttrium acetate, not all of the yttrium was observed

to be taken up as the starburst chelate, the acetate
carboxyl resonance was observed to shift to 183.8 ppm
indicating that some of the yttrium was associated with
the acetate. The integrated area of the chelated $-CH_2$

5   groups on the dendrimer increased, indicating that some
of the third mole equivalent of yttrium added was
indeed chelated with the dendrimer. These results
indicate that the dendrimer can chelate from 2-3
yttrium ions per dendrimer molecule.

10.

Example 6: Demonstration of Multiple Chelation of
Yttrium by a methylene carboxylate terminated second
generation starburst polyamidoamine by trans chelation
from yttrium acetate.

15   The same experimental methods were used for
this study as were used for Example 5. The starburst
polyamidoamine methylene-carboxylate terminated
material (0.40g 62.5% active, remainder sodium bromide,
0.12 mmol.) was dissolved in 4-5 ml of deuterium oxide.

20   The resultant pH was 11.5-12, which was lowered to 9.4
with 6N HCl prior to the experiment. A solution of
yttrium acetate was prepared by dissolving yttrium
chloride (0.1125g, .37 mmol.) and sodium acetate

25   (0.0915g, 1.1 mmol.) in 1.5 ml of deuterium oxide, thus
every 0.5 ml of solution contains one mole equivalent
of metal.

The first two mole equivalents of yttrium
30   acetate added were fully chelated by the starburst
polyamidoamine. On addition of a third mole equivalent
of yttrium, precipitation of the product occurred and
as such no NMR data could be obtained. The signals
which gave the most information about chelation by the
35   starburst dendrimer were those of the two carbons
adjacent to the chelating nitrogen. The chemical

shifts of these carbons in the unchelated dendrimer occurred at 59.1 ppm for the α-CH_2, and 53.7 ppm for the first methylene carbon of the backbone. Upon chelation these two resonances were observed to shift

5   downfield to 60.8 and 55.1 ppm respectively. The trans chelation shows that two metal ions can be readily chelated per dendrimer molecule, however upon chelation of some unknown fraction of a third mole equivalent, the product precipitates out of solution.

10

Example 7: Demonstration of Multiple Chelation of ^{90}Y by a methylenecarboxylate terminated second generation starburst polyethyleneimine.

15   Standard solution of yttrium chloride (3×10^{-2} M, spiked with non-carrier added ^{90}Y) and methylenecarboxylate terminated second generation starburst polyethyleneimine (6×10^{-2} M) were prepared. These were reacted together at various metal:starburst

20   ratios in HEPES buffer. The complex yield was determined by ion exchange chromatography using Sephadex G50 ion exchange beads, eluting with 10% $NaCl:NH_4OH$, 4:1 at pH 10. Noncomplexed metal is removed on the column, complexed metal elutes. Yields

25   were obtained by comparing the radioactivity eluted with that on the column, using a well counter.

30

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Table V

Chelation of 2.5 Gen. PEI Acetate with ^{90}Y

Vol. Y+3	Vol. PEI	Vol HEPES	M:L Theor.	% Complex	M:L Act.
5	30	370	0.1	110	0.1
10	30	360	0.2	101	0.2
20	30	350	0.4	95	0.4
30	35	340	0.5	97	0.5
30	30	340	0.5	102	0.5
60	30	310	1.0	99	1.0
120	30	250	2.0	100	2.0
180	30	180	3.0	94	2.8
250	30	120	4.1	80	3.3
300	20	80	7.5	44	3.3
300	20	70	5.0	40	2.0
300	20	70	5.0	41	2.0

All volumes in Table V are in microlitres

Within the accuracy of the experiments, these results indicate that the 2.5 Gen. starburst PEI acetate can chelate between 2 and 3 metals per polymer giving a soluble complex.

Example 8: Demonstration of multiple chelation of iron by a sodium propionate terminated sixth generation starburst polyamidoamine.

The sodium propionate terminated sixth generation polyamidoamine (initiated from ammonia) (97.1 mg, 2.45 mol.) was dissolved in 1.5 ml of deionized water. Addition of 0.5 ml of 0.5N HCl

reduced the pH to 6.3. Ferric chloride was added (0.5
ml of 0.1.2M solution, 0.051 mmol) producing a light
brown gelatinous precipitate. On heating at 60°C for
0.5 hours, the gelatinous precipitate became soluble,
resulting in a homogeneous orange solution. The
solution was filtered through Biogel P2 acrylamide gel
(10 g, twice) isolating the orange band (free of halide
contamination). Removal of the solvent _in vacuo_ gave
the product as an orange film (30 mg). Analysis was
consistent with chelation of approximately 20 moles of
ferric ions per mole of starburst dendrimer.

Table IV

Found	Theoretical		
	$Na_4Fe_{20}H_{128}SB$	$Na_5Fe_{20}H_{127}SB$	$Na_6Fe_{20}H_{126}SB$
Na 0.39,0.24 (0.31 0.1%)	0.25	0.31	0.38
Fe 3.14,3.11 (3.12 0.02%)	3.05	3.05	3.04
C 47.11	49.87	49.84	49.81
H 7.33	7.31	7.30	7.29
N 14.81	14.49	14.48	14.47
O ----	25.03	25.02	25.01
Mwt.	36632.23	36654.21	36675.13

$$SB = C_{1521}H_{2467}N_{379}O_{573}$$
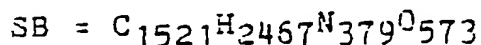
These results confirm chelation of 20 ± 2 moles
of ferric ions per mole of starburst dendrimer.

WE Claim:

1.   A starburst conjugate which comprises at least one starburst polymer associated with at least one unit of at least one carried agricultural material.

2.   The conjugate of Claim 1 wherein the starburst polymer is a starburst dendrimer.

5

3.   The conjugate of Claim 1 or 2 wherein at least one of the carried agricultural materials is a pesticide, radionuclide, chelant, toxin, signal generator, signal reflector, or signal absorber.

10

4.   The conjugate of Claim 2 wherein there are at least two different carried materials at least one of which is a target director and at least one of which is a bioactive agent.

15

5.   The conjugate of Claim 4 wherein the target director is an entity specific for one or more target receptors and the bioactive agent is a radionuclide, pesticide or toxin.

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6. The conjugate of Claim 1 wherein the dendrimer contains discontinuities.

7. A starburst conjugate of claim 1 the
5 formula:

$$(P)_x * (M)_y \qquad (I)$$

wherein each P represents a dendrimer;

10 x represents an integer of 1 or greater;

each M represents a unit of a carried agricultural material, said carried agricultural material can be the same carried agricultural material or a different
15 carried agricultural material;

y represents an integer of 1 or greater; and

* indicates that the carried agricultural material is
20 associated with the dendrimer.

8. The conjugate of Claim 7 wherein M is a pesticide, radionuclide, chelator, chelated metal, toxin, signal generator, signal reflector, or signal
25 absorber.

9. The conjugate of Claim 7 wherein x=1 and y=2 or more.
30

10. The conjugate of Claim 8 wherein y=2 or more.

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11. The starburst conjugate of Claim 7 wherein the molar ratio of any ionic M to P is 0.1-1,000:1.

12. The starburst conjugate of Claim 9 wherein the weight of the ratio of any pesticide or toxin M to P is 0.1-5:1.

13. A process for preparing
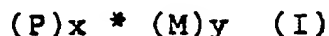
$$(P)x * (M)y \quad (I)$$

wherein each P represents a dendrimer; x represents an integer of 1 or greater; each M represents a unit of a carried agricultural material, said carried agricultural material can be the same carried agricultural material or a different carried agricultural material; y represents an integer of 1 or greater; and * indicates that the carried agricultural material is associated with the dendrimer, which comprises reacting P with M, usually in a suitable solvent, at a temperatue which facilitates the association of the carried agricultural material (M) with the starburst dendrimer (P).

14. The process of Claim 13 wherein the temperature is from room temperature to reflux.

15. The process of Claim 13 wherein the suitable solvent is water, methanol, ethanol, chloroform, acetonitrile, toluene, dimethylsulfoxide or dimethylformamide.

16. A starburst conjugate composition which comprises one or more starburst conjugates of any one of Claims 1-12 and at least one agriculturally acceptable diluent or carrier.

17. The starburst conjugate composition of Claim 16 which also contains other active ingredients.

18. A starburst conjugate composition of any one of Claims 1-12, 16 or 17 for use as an _in vivo_ or _in vitro_ diagnostic compound.

19. A starburst conjugate composition of any one of Claims 1-12, 16 or 17 for use as an _in vivo_ therapeutic compound.

20. A method for the delivery of at least one carried agricultural material which comprises administering of at least one starburst conjugate, as defined in Claims 1-12, 16 or 17 containing said material at or near a targeted locus.

21. A method for scavenging therapeutic or diagnostic compounds which comprises _in vivo_ administration of a bifunctional starburst conjugate of any one of Claims 1-12, 16 or 17 containing a target director which localizes the conjugate to a target locus and a scavenging moiety which can bind a secondarily administered therapeutic or diagnostic compound.

22. The method of Claim 21 wherein the scavenging moiety is a chelant.

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1.   A starburst conjugate which comprises at
least one starburst polymer associated with at least
one unit of at least one carried agricultural material.

2.   The conjugate of Claim 1 wherein the
starburst polymer is a starburst dendrimer.

5
3.   The conjugate of Claim 1 or 2 wherein at
least one of the carried agricultural materials is a
pesticide, radionuclide, chelator, chelated metal,
toxin, signal generator, signal reflector, or signal
10   absorber.

4.   The conjugate of Claim 2 wherein there are
at least two different carried materials at least one
of which is a target director and at least one of which
15   is a bioactive agent.

5.   The conjugate of Claim 4 wherein the
target director is an entity specific for one or more
target receptors and the bioactive agent is a
20   radionuclide, pesticide or toxin.

25

22.   The method of Claim 21 wherein the scavenging moiety is a chelant.

23.   The conjugate of Claim 2 wherein the starburst dendrimer is of the formula

$$\left(Core \right) \left\{ (Repeat\ Unit)_{\frac{N_r^{G}-1}{N_r-1}} \quad \begin{matrix} Terminal \\ Moiety \end{matrix} \quad N_r^{G} \right\}_{N_c}$$
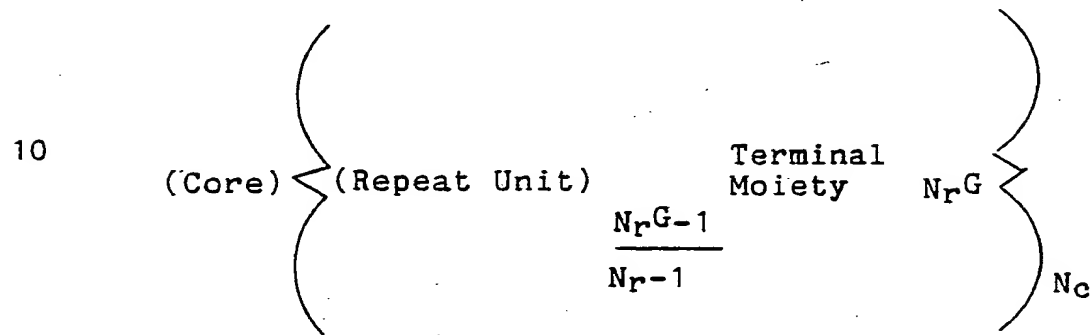
wherein: the core is

\# of terminal groups per dendritic branch =

$$\frac{N_r^{G}}{2} \quad ;$$

G is the number of generations; N_r is the repeating unit multiplicity which is at least 2; N_c is the valency of the core compound; the terminal moiety is determined by the following:

\# of terminal moieties per dendrimer =

$$\frac{N_c N_r^{G}}{2}$$

wherein N_r, G and N_c are as defined above; and the Repeat Unit has a valency or functionality of $N_r + 1$ wherein N_r is as defined above.

24. The conjugate of Claim 2 wherein the starburst dendrimer is of the formula

$$\left(\left(\boxed{I} \right) (Z^c)_{N_c} \right) \lessgtr \left(\left(X^i Y^i (Z^i)_{N^i} \right)^{\substack{i-1 \\ N_c \pi N^n \\ n \text{ is } 1}} \right) \lessgtr \left(X^t Y^t (Z^t)_{N^t} \right)^{\substack{t-1 \\ N_c \pi N^n \\ n \text{ is } 1}}$$
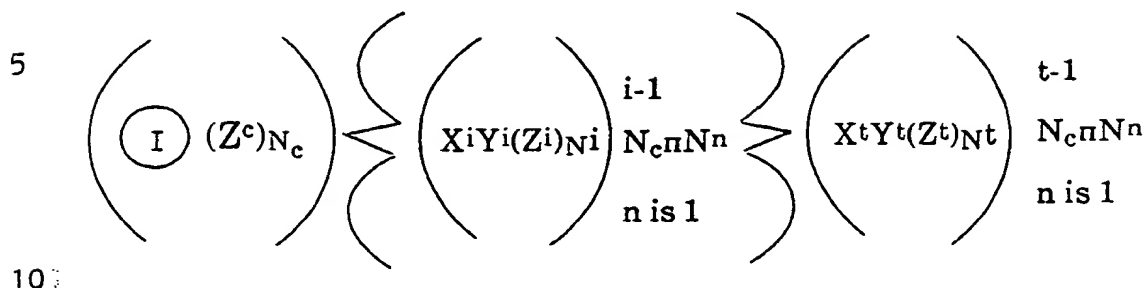
wherein i is 1 to t-1; the core compound is represented by the formula

$$\boxed{I} \ (Z^c)_{N_c}$$

where

$$\boxed{I}$$

represents the core, Z^c represents the functional groups bonded to
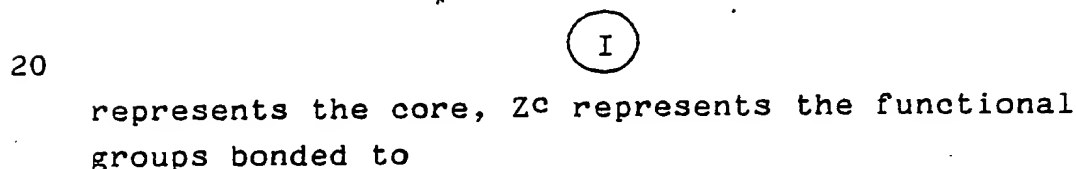
$$\boxed{I}$$

and N_c represents the core valency; the repeat unit is represented by the formula $X^i Y^i (Z^i)_{N^i}$ wherein "i" is defined as above; the final or terminal units are represented by $X^t Y^t (Z^t)_{N^t}$ wherein t represents terminal generation and X^t, Y^t, Z^t and N^t may be the same as or different from X^i, Y^i, Z^i and N^i except that there is no succeeding generation connected to the Z^t groups and N^t may be less than two; the n function is the product of all the values between its defined limits, such as

$$\Pi_{n=1}^{i-1} N_n = (N^1)(N^2)(N^3)...(N^{i-2})(N^{i-1})$$

which is the number of repeat units, $X^i Y^i (Z^i)_{N^i}$, comprising the ith generation of one dendritic branch and when i is 1, then $\Pi_{n=1}^{o} = 1$.

25. A process for preparing a starburst conjugate as defined in Claim 1 which comprises the reaction of P, having reactive moieties, with an aniline moiety, which may have the NH_2 group protected by an N-phthalimide of the formula
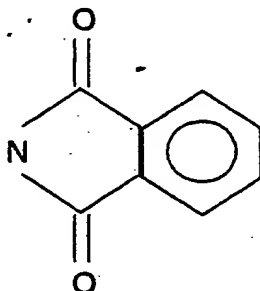
26. A process for preparing a starburst conjugate as defined in Claim 1 which comprises the reaction of P, having reactive moieties, which may have the NH_2 group protected by any protecting group used for amines which is inert under the conditions used for starburst synthesis.

27. A process for preparing a starburst polyethyleneimine which comprises reacting a starburst polyethyleneiminemethane sulfonamide with hydrochloric acid.

28. A process for purifying a starburst dendrimer having a solvent present which comprises removing the solvent by ultrafiltration using a membrane.

5

29. The process of Claim 28 wherein the solvent is ethylenediamine.
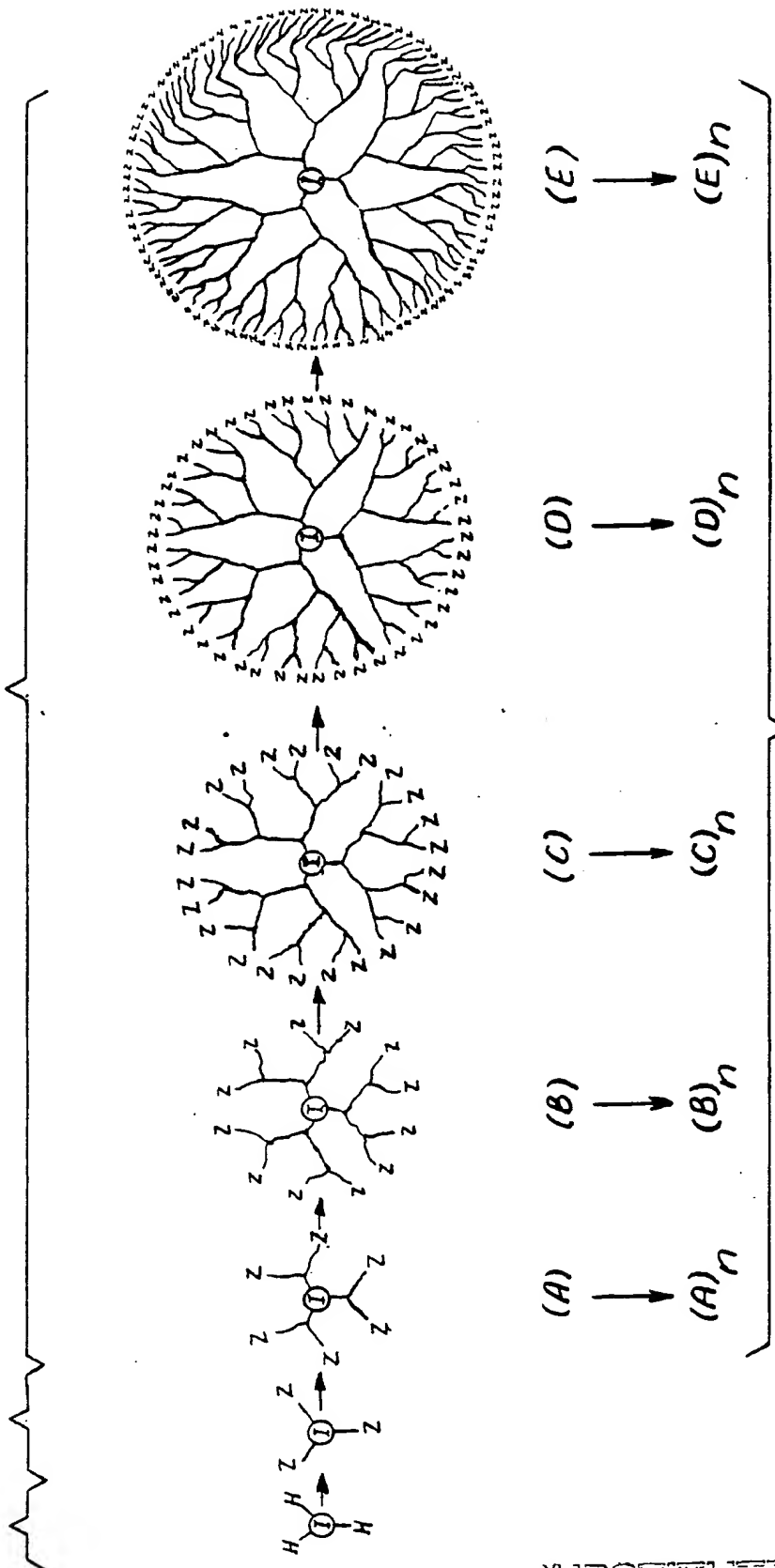
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FIG. 3

Generation of added PAMAM Starburst Dendrimer

Fig. 2

(A)

(B)

Fig. 1
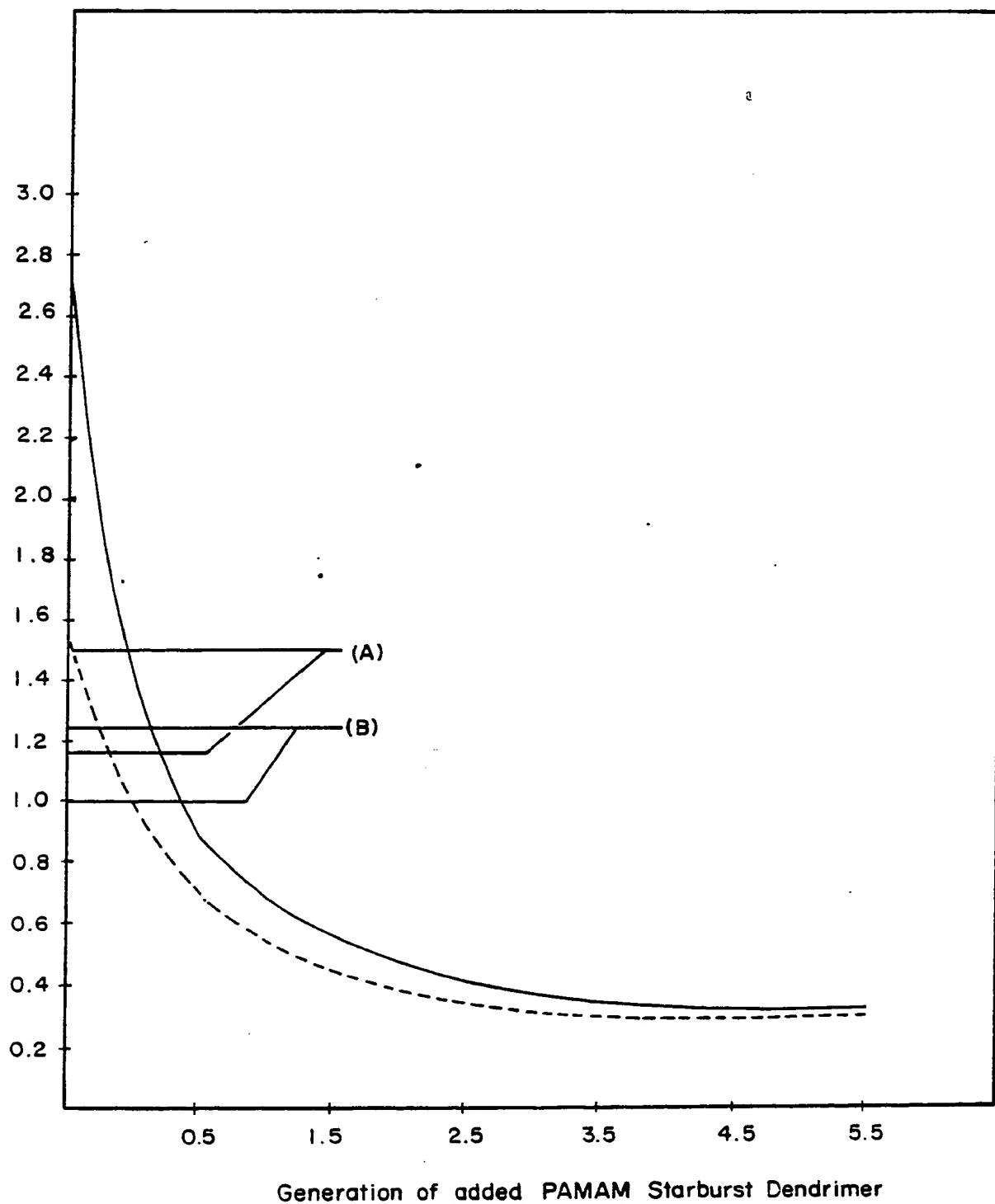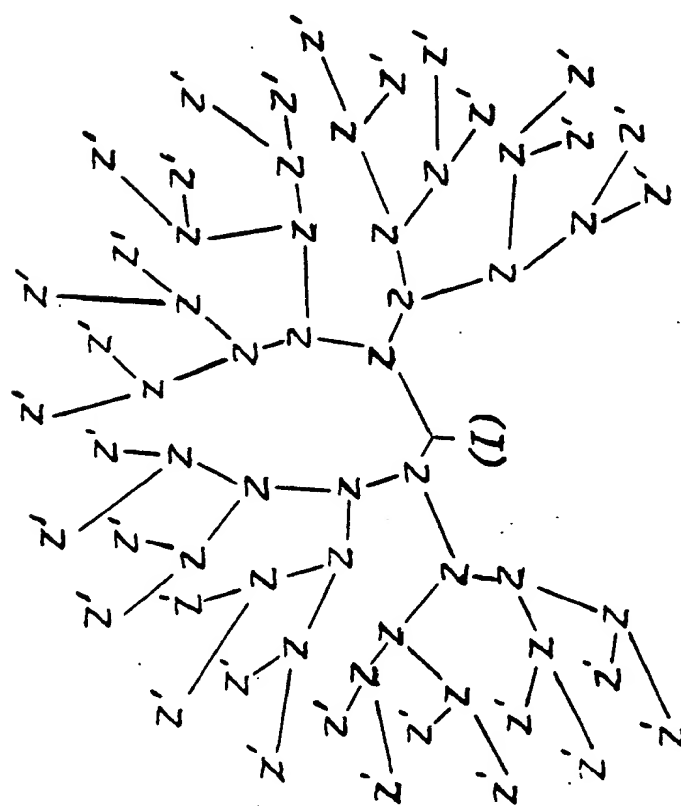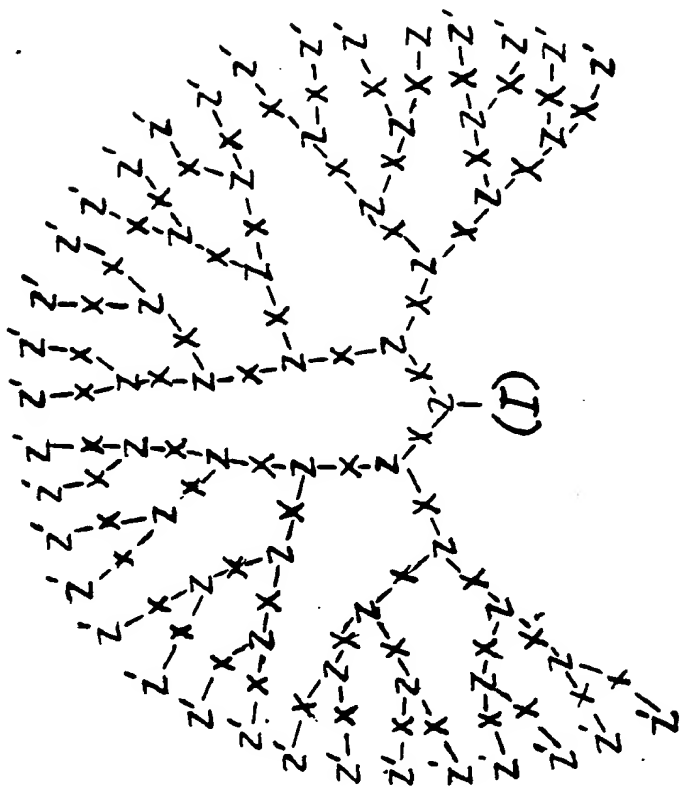
INTERNATIONAL SEARCH REPORT

International Application No PCT/US 87/02075

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

INT CL.4 A 61K 49/02
US CL. 424/1.1

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
U.S.	424/1.1 ,9 525/410,416,451, 418 528/310, 332, 350, 363, 397

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched [6]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category [*]	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
X Y	US, A, 4,558,120 PUBLISHED 10 December 1985 Tomalia (col. 12, lines 16-14)	1, 2, 6-13 3, 14, 15
P, A	US, A, 4,606,907 PUBLISHED 19 August 1986 Simon et al.	
T	US, A, 4,694,064 PUBLISHED 15 September 1987 Tomalia et al.	

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

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IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
29 October 1987	2 5 NOV 1987

International Searching Authority [1]	Signature of Authorized Officer [20]
ISA/US	John S. Maples

Form PCT/ISA/210 (second sheet) (May 1986)

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